

[COMMITTEE PRINT]

ANIMAL SCIENCES: ADVANCES IN REPRO-
DUCTIVE AND HEALTH TECHNOLOGIES

REPORT

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FOR THE
SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT

TRANSMITTED TO THE
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LETTER OF TRANSMITTAL

HOUSE OF REPRESENTATIVES,
COMMITTEE ON SCIENCE AND TECHNOLOGY,
Washington, D.C., September 26, 1983.

HON. DON FUQUA,
*Chairman, Committee on Science and Technology,
U.S. House of Representatives.*

DEAR MR. CHAIRMAN: I am pleased to transmit to you a report recently completed for the Subcommittee on Investigations and Oversight by the Congressional Research Service entitled *Animal Sciences: Advances in Reproductive and Health Technologies*.

For some years now the Investigations and Oversight Subcommittee has taken a strong interest in scientific development affecting agriculture. Last Congress the Subcommittee held hearings on potential applications of advances in recombinant DNA and genetics on agricultural sciences. At those hearings, the Subcommittee heard from leading experts in academia, industry and government about the rapid progress of science in certain areas of livestock breeding, such as twinning and embryo transplantation and about the difficult tasks remaining as the scientific community sought to further unravel the mysteries of animal and plant genetics.

Because of the potentially enormous impact of genetic engineering and other scientific advances in the agricultural sector, the Subcommittee felt it would be useful to have the Congressional Research Service prepare this summary for both lay and scientific readers. We anticipate that the report will have a wide audience in the Members of the Committee on Science and Technology and other Members of Congress, and in Farm Bureaus, 4-H clubs, universities, and farm communities throughout the Nation.

I would like to express my appreciation to Jim McCullough, Chief, Science Policy Research Division, and Judith Johnson, Analyst in Life Sciences, both of the Congressional Research Service, and to Stephanie Forbes, for their time and excellent work in preparing this report.

Sincerely,

ALBERT GORE, JR.,
*Chairman,
Subcommittee on Investigations and Oversight.*



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LETTER OF SUBMITTAL

July 29, 1983

Honorable Albert Gore, Jr.
Chairman, Subcommittee on
Investigations and Oversight
Committee on Science and Technology
House of Representatives
Washington, D.C. 20515

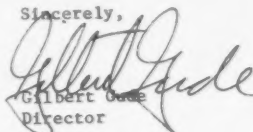
Dear Mr. Chairman:

I am pleased to submit the report entitled "Animal Sciences: Advances in Reproductive and Health Technologies," prepared at the request of the Subcommittee on Investigations and Oversight of the House Committee on Science and Technology.

This document discusses recent advances in animal science, concentrating on two major areas, reproduction and health. This project was prepared by Judith A. Johnson, Analyst in Life Sciences, with assistance provided by Stephanie Forbes.

On behalf of the Congressional Research Service, I thank you for the opportunity to assist your subcommittee in the preparation of this report.

Sincerely,


Gilbert Gade
Director

Enclosure

(A)



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ANIMAL SCIENCES: ADVANCES IN REPRODUCTIVE AND HEALTH TECHNOLOGIES

Judith A. Johnson
Analyst in Life Sciences
Science Policy Research Division
July 28, 1983

(VII)

CONTENTS

OVERVIEW.....	1
I. REPRODUCTIVE TECHNOLOGIES.....	3
A. <u>ARTIFICIAL INSEMINATION</u>	3
<u>Use in Cattle Production</u>	3
<u>Dairy Cattle</u>	6
<u>Beef Cattle</u>	9
<u>Sheep Industry</u>	9
<u>Dairy Goat Industry</u>	9
<u>Pork Industry</u>	10
<u>Horse Breeding</u>	10
<u>Poultry Industry</u>	11
<u>Economic Impact on Cattle Industry</u>	11
B. <u>EMBRYO TRANSFER INDUSTRY</u>	12
<u>Superovulation</u>	14
<u>Embryo Collection</u>	16
<u>Embryo Culture/Storage/Cryopreservation</u>	18
<u>Estrus Detection/Synchronization</u>	19
<u>Embryo Transfer</u>	20
<u>Applications of Embryo Transfer</u>	22
<u>Economic Impact of Embryo Transfer</u>	24
C. <u>SEXING OF SPERM AND EMBRYOS</u>	25
D. <u>IN-VITRO FERTILIZATION</u>	27
E. <u>PARTHENOGENESIS, CHIMERAS, TWINNING AND CLONING</u>	28
F. <u>NUCLEAR TRANSPLANTATION</u>	31
<u>Amphibians</u>	31
<u>Mammals</u>	32
<u>Applications</u>	33

(IX)

G.	<u>GENE TRANSFER</u>	34
	<u>Methods</u>	35
	<u>Applications</u>	37
II.	<u>HEALTH TECHNOLOGIES</u>	38
A.	<u>VACCINE PRODUCTION</u>	38
	<u>Viral Vaccines</u>	44
	<u>Foot and Mouth Diseases</u>	44
	<u>Rabies</u>	47
	<u>Parvovirus</u>	49
	<u>Bovine Viral Diarrhea</u>	50
	<u>Papillomavirus</u>	51
	<u>Rift Valley Fever</u>	52
	<u>Bluetongue</u>	52
	<u>Transmissible Gastroenteritis</u>	54
	<u>Bacterial Vaccines</u>	55
	<u>Colibacillosis</u>	55
	<u>Mastitis</u>	57
	<u>Protozoal/Parasitic Vaccines</u>	58
	<u>Coccidiosis</u>	58
	<u>Trypanosomiasis</u>	59
	<u>Malaria</u>	60
	<u>Babesiosis</u>	61
B.	<u>INTERFERONS</u>	63
C.	<u>DIAGNOSTICS/MONOCLONAL ANTIBODIES</u>	64
D.	<u>ANIMAL HORMONES</u>	64
	<u>Growth Hormone</u>	65
	<u>Prolactin</u>	66
	<u>Hormone Delivery and Slow-Release Technology</u>	67
E.	<u>ANIMAL NUTRITION</u>	68
	<u>Feed Grain Improvement</u>	68
	<u>Feed Additive Production--Amino Acids</u>	71
	<u>Single Cell Protein</u>	73
	<u>Manipulation of Intestinal Microflora</u>	74
	<u>CONCLUSION</u>	75

OVERVIEW

Man has evolved in an environment containing other animal species which he depends on for food, clothing, recreation, and companionship. Animal products for human consumption provide over 98 percent of the vitamin B₁₂, in the average human diet, as well as 69 percent of the protein, 80 percent of the calcium, 67 percent of the phosphorus and vitamin B₆, over 50 percent of the vitamin B₁₂ and a substantial proportion of most other nutrients. ^{1/} The survival of human societies without their domestic animals would certainly be difficult, if not impossible, especially in nonaffluent cultures. ^{2/}

As specified by the subcommittee, this paper is limited to recent advances in animal science and concentrates on two major areas: reproduction and health. The section on reproductive technologies begins with a discussion of artificial insemination, which has been used as an animal breeding tool in the United States since 1938. The impact of artificial insemination upon livestock productivity, particularly in the dairy industry, may serve as a model for the possible impact of the remaining techniques discussed in the section, which are not as highly developed. These techniques include embryo transfer, sexing of sperm and embryos, in vitro fertilization, twinning, cloning, nuclear transplantation and gene transfer.

^{1/} Pond, Wilson G., et al. *Animal Agriculture: Research to Meet Human Needs in the 21st Century*. Boulder, Colorado, Westview Press, 1980. p. 36.

^{2/} Seidel, George E., Jr., and Benjamin G. Brackett. *Perspectives on Animal Breeding*. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. *New Technologies in Animal Breeding*. New York, Academic Press, 1981. p. 4.

The second section discusses recent advances in biotechnology that hopefully will improve animal health. Diseases affecting livestock can cause major economic losses around the world and reduce food production at a time when both population and the demand for meat are growing. African cattle farmers, for example, lose \$5 billion a year because large areas of land are infected with trypanosomiasis, or sleeping sickness, and cannot be used for cattle grazing.

Advances in biotechnology offer the promise of solving some of the current problems in animal health care. These advances, including the production of recombinant DNA and synthetic peptide vaccines to prevent disease, the development of monoclonal antibodies to diagnose infection and offer passive immunity, the creation of improved feed crops and additives to improve animal nutrition, and the large-scale manufacture of growth hormones to speed animal maturation, should help the farmer produce more food for more people.

I. REPRODUCTIVE TECHNOLOGIES

A. ARTIFICIAL INSEMINATION

In 1938, the technique of artificial insemination (AI) was introduced to the United States from Europe where it had been used primarily to control venereal disease in livestock. AI use in the United States, however, has mainly focused on the genetic improvement of domestic animals. For example, the U.S. dairy herd now numbers less than half what it was in 1950, yet it produces more milk. AI techniques are more highly developed for use in cattle than in other livestock species, and for a variety of reasons AI is much more frequently used in dairy cows than in beef cattle. Of the 11 million commercial dairy cows in the United States today, about 60 percent have been bred by AI. In contrast, of the 80 million beef cattle in the United States, only 5 percent have been bred using AI. ^{3/} This section will focus on the use of artificial insemination techniques in cattle and will also briefly describe its use in other livestock species.

Use in Cattle Production

There are many advantages to using AI in livestock reproduction, the greatest being the genetic improvement possible through careful sire selection. A bull in natural service may breed 25 cows per year over a lifetime of approximately 8 years, producing possibly 200 offspring. When AI is used, a bull,

^{3/} Caras, Roger. Breeding A Revolution. Geo, v. 2, Aug. 1980. p. 85.

CRS-4

whose sperm is collected three times weekly over an 8-year period with each collection extended to breed approximately 200 cows, could ultimately produce 249,600 offspring. This conservative estimate is over 1,000 times what is expected from natural service. AI also allows for the rapid identification of high-quality sires. This may be accomplished in a single generation where it would usually take a lifetime to accumulate the same information using natural service. AI allows for a more complete and accurate record of livestock crosses and the resultant progeny's genetic worth. Such records are necessary for good reproductive management of a livestock herd and, when reported to central stations for processing, they provide valuable information for fertility and genetic research. AI can be used to overcome problems of breeding incompatibility that are due to, for example, injury, physical size, age, temperament or other factors. It also eliminates the need for the presence of dangerous bulls on dairy farms; dairy bulls are usually more excitable than beef bulls and must be handled with great caution. The use of AI has resulted in a significant reduction in the frequency of recessive lethal genes. Its use has also been instrumental in the control of certain diseases, especially venereal diseases. And AI is economical to use. The cost of using an outstanding sire through AI is less than through natural service and the value of the progeny produced exceeds the costs of semen and service. Finally, the success of AI as a breeding technique has provided the basis for the development of many other potentially useful techniques, such as in vitro fertilization and embryo transfer, which will be discussed in the following sections.

Bull semen is frequently collected using an artificial vagina, or AV. The AV is a stiff rubber hose which has been fitted with a soft rubber liner. Between the hose and the liner is a space containing warm water. One end of the AV is open and well greased, and at the other end is a flexible rubber

CRS-5

funnel which is attached to a collecting test tube. The bull is allowed to mount another animal, usually a castrated male, and a technician using the AV can collect the semen in less than 5 seconds. Alternatively, an electro-ejaculator may be used, eliminating the need for a mount-animal and enabling semen collection from injured and impotent animals and those unaccustomed to handling. Bull semen is collected two or three times a week, usually two ejaculations 10 or 15 minutes apart, yielding 10 to 30 milliliters of semen. The sample is examined under a microscope for sperm motility and with a spectrophotometer for sperm concentration.

A number of technical advances have occurred over the last 40 years in the collection, processing and storage of semen. Through the study of sexual behavior, methods of sexual preparation have been developed, along with an optimal schedule of semen collection. These efforts have resulted in an increase in the number of sperm cells harvested per sire per week from about 8-10 billion to about 30-40 billion. ^{4/} Methods of extending (or diluting) semen and preserving sperm also have been developed which permit more inseminations per ejaculation.

Following the sample's evaluation for sperm motility and density, it is diluted and cooled to 41 degrees F. After 3 to 4 hours, glycerin is added as an antifreeze to prevent the formation of harmful ice crystals which would rupture the sperm when the sample is later frozen. Next the sample is divided into .5 to 1 milliliter quantities and packaged in small plastic straws or glass ampules. Each straw or ampule contains from 10-15 million sperm, enough for one insemination. The straws or ampules are then frozen at -385 degrees F in liquid nitrogen. The freezing process kills up to one-half of the sperm cells

^{4/} Foote, Robert H. The Artificial Insemination Industry. In: Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 19.

CRS-6

and tends to lower the fertility of the surviving cells slightly. However, the decrease in fertility is considered to be offset by the increased convenience of frozen semen, which may be stored indefinitely.

Before a cow is inseminated, it is necessary to determine if she is in estrus. Estrus is defined as the time during which the female will accept the services of the male and is capable of conceiving. It is cyclical in nature, the length and period varying among species. The cow is considered to be a polyestrous animal because she cycles continuously. (Estrus detection will be discussed in greater detail in the following section on embryo transfer.)

The most common technique for artificially inseminating a cow is the rectocervical method. The semen, which had previously been frozen in a straw or ampule, is thawed and inserted into the inseminator. A technician inserts one arm into the rectum of the cow and directs the inseminating tube through the vagina and cervix for deposition of the semen in the anterior cervix. Provided the cow was in estrus and the insemination was performed properly, a calf should be expected in approximately 283 days.

Dairy Cattle

The use of AI in the U.S. dairy industry is largely responsible for the increased milk production per cow mentioned at the beginning of this section. Between 1945 and 1975 the average amount of milk produced per dairy cow per year increased from approximately 2,000 kilograms (kg) to 4,700 kg. ^{5/} In 1945, 26.6 million dairy cows produced 54 billion kg of milk; in 1975,

^{5/} Pond, Wilson G., et al. *Animal Agriculture: Research To Meet Human Needs in the 21st Century*. Boulder, Colorado, Westview Press, 1980. p. 117.

CRS-7

11.6 million cows produced 55 billion kg of milk. 6/ The feed that would have been required for maintenance of the 15 million cows which was not needed in 1975 is essentially feed saved and has been estimated at more than 1,000,000,000 bushels of corn annually. 7/ The increased milk production per cow has also resulted in decreased labor costs and facilities requirements.

The genetic improvement due to AI use in dairy cows is permanent and has been estimated to account for approximately 30 percent of the total increase in milk production. 8/ The balance is due to environmental factors such as feeding, housing and management. The importance of genetic superiority in dairy cows can be appreciated when one considers the fact that 400 pounds of blood must pump through a cow's udder for every pound of milk she produces. 9/ Thus, the construction of the udder, which is genetically determined, is of great importance. Superior dairy sires are usually identified by the milk production of their sister siblings and, of course by the milk production of their female progeny. Superior dairy cows average over 10,000 kg of milk per year with the record production of a single cow being over 25,000 kg of milk in one year. 10/

The National Cooperative Dairy Herd Improvement Program (NCDHIP) is a nationwide program for collecting, analyzing and disseminating information on dairy cattle performance. On the local level, the Dairy Herd Improvement

6/ Ibid.

7/ Foote, Artificial Insemination Industry, p. 29.

8/ Ibid., p. 22.

9/ Caras, Breeding A Revolution, p. 88.

10/ Warwick, Everett James, and James Edward Legates. Breeding and Improvement in Farm Animals. McGraw Hill, 1979. p. 348.

Association (DHIA) collects performance data on each animal's milk production from the individual dairies. This information is sent to the Animal Improvement Programs Laboratory (AIPL) at USDA in Beltsville, Maryland which analyzes and incorporates it into the "USDA-DHIA Sire Summary List," published biannually for public use. The list is used by dairy breeders in selecting superior sires. NCDHIP is a cooperative program, financed largely by the dairy industry. The data provided by the program is considered to be impartial, since it is collected by disinterested DHIA officials and processed under high security conditions. AIPL's sire summaries are respected internationally because of the absence of commercial influence on their compilation and analysis of milk production data.

Not every State or region participates in the NCDHIP. In areas where dairying is scarce or only a part-time operation, a strong local DHIA does not exist. However, dairy cows enrolled in the the NCDHIP consistently outproduce the nonenrolled cows. In the May 1, 1977 to April 30, 1978 testing year, the NCDHIP animals produced 5,000 pounds more milk per cow, representing 52 percent more milk per lactation. ^{11/} The genetic gain that is due to the use of AI is predicted to continue increasing. There is no evidence at the present time that genetic variability is decreasing or that a plateau in milk production improvement has been reached.

The use of AI has been greater in dairy cattle than in other species because of their high value, regular contact with dairymen, the use of confined housing and the good results obtained with the use of frozen semen. Furthermore, milk yield and fat content are obvious traits for selection in dairy cattle. In other livestock species, the selection traits are not as obvious.

^{11/} U.S. Congress. Office of Technology Assessment. Impacts of Applied Genetics. Washington, U.S. Govt. Print. Off., 1981. p. 181.

Beef Cattle

Although the beef cattle industry is much larger than the dairy industry in terms of numbers of animals and their dollar value, less than 5 percent of its animals have been bred using AI. ^{12/} Natural stud service is usually more economical. Estrus detection in large numbers of cows scattered widely on the range is a significant barrier to AI application in beef cattle. Confined housing and the use of effective methods in estrus regulation would eliminate this problem.

Sheep Industry

Test programs using AI in sheep have not been particularly successful. The problems of handling animals on the range and difficulties in estrus detection remain as major barriers to AI application in the sheep industry. The use of AI is also limited by the poor fertility of frozen ram semen and relatively high labor costs per inseminated animal. The low cost of performance-tested rams available for natural service remains preferable to AI.

Dairy Goat Industry

The dairy goat industry has recently become interested in AI and has formed the "Coordinating Sub-Group for Dairy Goats" of the NCDHIP. Although dairy goats are housed in confined areas and AI is technically feasible, the insemination procedure is more difficult and the value per animal, labor costs, and estrus detection problems limit the potential application of AI.

^{12/} Foote, The Artificial Insemination Industry, p. 18.

Pork Industry

The increased use of confined housing in swine production has made AI more attractive to the pork industry. Although estrus detection is still a problem with gilts (young female swine), estrus in sows may be synchronized by the simultaneous weaning of litters. The fertility of frozen boar semen has been low, and when the female does conceive, the litter size tends to be smaller. Fresh semen generally gives better results when AI is used in swine. However, tested boars are available for natural service at relatively low cost. The concentration of sperm in boar ejaculate is very low and requires additional processing before freezing. The processing of boar semen still needs further research before a feasible AI method will become available for widespread commercial use in swine.

Horse Breeding

Horses are relatively easy to breed with AI because of size and anatomical structures. However, certain horse breed organizations are opposed to the use of AI and do not allow the registration of animals which have been bred using AI. As a result, commercial attempts to offer AI services to horse breeders have not been successful. Conception rates with frozen stallion semen are low; therefore, when artificial insemination is used on mares, fresh semen is usually employed. The period of estrus in the mare is considerably longer than in other species, lasting usually 6 to 8 days. (Estrus duration in the cow, ewe and sow is 16 hours, 30 hours and 44 hours, respectively.) Detection of ovulation in such a long estrous period is difficult and often necessitates multiple inseminations. AI can be used along with natural service for multiple inseminations in order to conserve the stallion. A stallion in high demand for natural service might otherwise exhaust his semen supply and conception rates would consequently decrease.

Poultry Industry

The entire turkey industry in the United States depends on AI since the selection of large amounts of white meat has produced birds which have difficulties in mating. Fresh semen is generally used in the poultry industry and can be rapidly collected. Frozen poultry semen is not as fertile and usually is not used. Large numbers of hens can be quickly inseminated using AI. Dilution methods for turkey semen have been developed, increasing the number of hens that may be mated to one male. Currently about 50 percent of hens are inseminated with diluted turkey semen. ^{13/}

Economic Impact on Cattle Industry

The use of AI and careful sire selection has made a definite economic impact upon the dairy industry. It has been estimated that for each \$1.00 invested in research, \$50.00 has been returned to the economy. ^{14/} Approximately 7 million dairy cows and heifers are inseminated annually. Dr. Robert H. Foote, Cornell University, estimates that in 1977 the genetic improvement of dairy cows had resulted in an increase of 1,000 pounds of milk per cow. ^{15/} Assuming that the price of milk is \$.10 per pound, Foote makes the following calculation: $7 \times 10^6 \text{ cows} \times 1000 \text{ lbs milk/cow} \times \$.10/\text{lbs milk} = \$700,000,000$. In other words, the increased genetic superiority of dairy cows was worth about \$700,000,000 in milk for the dairy industry in 1977.

^{13/} U.S. Congress, Office of Technology Assessment, Impacts of Applied Genetics, p. 190.

^{14/} Foote, The Artificial Insemination Industry, p. 22.

^{15/} Ibid.

CRS-12

The cost of bull semen varies from a minimum of \$3.00 per insemination to a few hundred dollars per insemination, the average being about \$8.00. The cost of providing professional insemination service is about \$5.00 per cow. ^{16/} Costs are approximately the same in the beef cattle industry. ^{17/}

West Virginia University's Allegheny Highlands Project (AHP) has emphasized the selection and use of superior weight-gaining bulls as herd sires (using natural mating or AI) in studies on improving beef cattle production. AHP also included improved nutrition, health and overall management in their efforts on increasing the calf weaning weight (a standardized measurement at 205 days of age, adjusted for the sex of the calf and the age of the calf's mother). Increases in calf weights at weaning have averaged 10 pounds per year of participation in AHP. ^{18/} It has been estimated that if the increase in calf weight achieved by AHP participants were extended to all the calves in the State, \$3,600,000 per year would be added to the income of West Virginia's beef producers when calf prices average \$50.00 per 100 pounds (hundredweight). If AI were used, this \$3,600,000 increased income could occur within 40 to 48 months. ^{19/}

B. EMBRYO TRANSFER INDUSTRY

Embryo transfer consists of removing an embryo in an early developmental stage from its genetic mother's reproductive tract and transferring it to

^{16/} Ibid., p. 31.

^{17/} Ibid., p. 35.

^{18/} Inskip, E. Keith, and John B. Peters. Economic Benefits of Reproductive Management. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, Inc., 1981. p. 246.

^{19/} Ibid.

CRS-13

another female's reproductive tract for development and subsequent birth. ^{20/}
In 1890, the first recorded embryo transfer was performed by Walter Heape, an English biologist, who succeeded in producing purebred Angoran rabbit young in a Belgian hare female. Few similar experiments were published until 50 years later, when embryo transfers in sheep were performed and reported. As one source notes, "A great amount of interest was generated in the late 1940s when livestock researchers felt . . . [embryo transfer] could be the female sequel of artificial insemination. Much--in fact too much--publicity was given to a program that did not materialize." ^{21/}

Embryo transfer continued to be a laboratory tool used primarily to study the process of reproduction until the early 1970s, when European breeds of cattle (Limousin and Simmental) became popular in the United States, Canada, Australia and New Zealand. ^{22/} The desired animals could not be imported because of strict quarantine regulations against foot and mouth disease. However, semen could be shipped into the United States, after a period of quarantine, from specific bulls that were under strict health restrictions. By using artificial insemination, the semen was then used to establish the desired European breed in the United States. A cow of any breed could be inseminated with semen from a bull of a desired foreign breed, for example, Simmental, and the resulting offspring would be one-half Simmental. A one-half Simmental heifer inseminated with purebred Simmental semen would produce a three-fourths Simmental offspring and its

^{20/} Sorensen, A. M., Jr. Animal Reproduction: Principles and Practices. McGraw-Hill, 1979. p. 344.

^{21/} Ibid.

^{22/} Seidel, George E., Jr., and Sarah M. Seidel. The Embryo Transfer Industry. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, Inc., 1981. p. 42.

CRS-14

mating to a purebred Simmental would result in a seven-eighths Simmental offspring. Most breed associations recognize three-fourths or seven-eighths animals as acceptable for breed recognition. Therefore, it would take three to four generations to produce "purebred" cattle by the above method, or about 5 to 7 years if everything went well, since gestation is about 9 months and a heifer should be at least 12 months old before breeding. While embryo transfer could not decrease the generation intervals, it could be used, along with superovulation, to produce multiple offspring from the desired three-fourths and seven-eighths "purebred" females. Cattle breeders provided the necessary economic incentives which influenced veterinarians and animal scientists into developing embryo transfer as a breeding tool.

The commercial application of embryo transfer is largely confined to the cattle industry. This section will primarily focus on the use of embryo transfer in cattle. The successful development of embryo transfer as an "industry" depended upon the concurrent development of several support technologies, including superovulation, embryo culture, cryopreservation of embryos, estrus detection and estrus synchronization. Each support technology will be discussed in the following subsections.

Superovulation

Embryo transfer programs are dependent upon the production of multiple ova. Since the cow usually only produces single or double ovulations, it must be stimulated to superovulate by injecting the animal with hormones such as pregnant mare's serum gonadotropin (PMSG), follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) and prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$). The exact mechanism of action for these hormone treatments is not understood at the present time.

CRS-15

About midway through the cow's estrous cycle, the animal is injected with PMSG, which causes the ovarian follicles to grow. ^{23/} Within each follicle is a developing egg, or ovum. Upon maturation of the follicle, it ruptures, releasing the ovum, which will then enter the oviduct. About 2 to 3 days after the PMSG treatment, the animal is injected with PGF_{2α} causing the premature lysis (disintegration) of the corpus luteum. The corpus luteum is the small mass of cells which develops at the site of each ruptured follicle. Estrus occurs about 2 days later and lasts for about 12 hours. Superovulated animals are then artificially inseminated (5 days after the first injection), since superior sires are usually available only through the use of AI. Multiple inseminations are used over a period of several hours since the eggs will not all be released from the ovary at the same time.

Superovulation protocols have been developed for several laboratory and farm animal species; each treatment series of hormone injections varies slightly, depending upon the species. According to George Seidel of the Animal Reproduction Laboratory of Colorado State University, "superovulation increases the number of normal ova recovered by about a factor of 10 for cattle, sheep, and goats but only by a factor of 2 or 3 for swine." ^{24/} Superovulation with artificial insemination in cattle usually results in 6 to 7 normal embryos and 2 to 3 abnormal embryos per cow. Three to four pregnancies after transfer will result. In 60 to 70 percent of cases of embryo collection without superovulation an ovum or embryo is recovered, 10 to 20 percent of which are unfertilized ova or morphologically abnormal embryos. "Thus, a normal embryo

^{23/} Seidel, George E., Jr. Superovulation and Embryo Transfer in Cattle. Science, v. 211, Jan. 23, 1981. p. 351.

^{24/} Seidel and Seidel, The Embryo Transfer Industry, p. 51.

CRS-16

is obtained 50 percent of the time. Pregnancy rates after transfer of such embryos are close to 70 percent." 25/

When cows are superovulated, variations in response to the hormone injections occur between different breeds of cattle, among different individuals of the same breed and between the first treatment and subsequent treatments of the same individual. 26/ Seasonal effects have also been demonstrated. 27/ Since superovulation results in greatly altered internal hormone levels for the animal, an interval of one to two complete estrous cycles between treatments is usually allowed to occur in order to avoid possible permanent alteration of the animal's natural cycle. 28/

The degree of success with superovulation cannot be predicted accurately for any individual animal; this is the greatest drawback of the technique. 29/ Preparations of PMSG and other hormones vary from batch to batch and are sometimes difficult to obtain. The FDA has recently declared PMSG a new animal drug; its use in the United States requires compliance with FDA regulations.

Embryo Collection

Following superovulation and artificial insemination, embryos are removed from the cow by using either surgical or nonsurgical methods. Surgery is used

25/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 354.

26/ Betteridge, K. J. Techniques and Results in Cattle: Superovulation. In Betteridge, K. J., ed. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 1.

27/ Ibid.

28/ Seidel and Seidel, The Embryo Transfer Industry, p. 51.

29/ Ibid., p. 52.

to recover embryos in the oviduct, and is the most efficient method for sheep, goats and pigs. Collection rates are higher and less scar tissue is formed when a mid-ventral (abdominal) incision is used rather than a flank incision. After locating the oviducts and uterus, the eggs are flushed from the animal's reproductive system into a collecting dish.

In the non-surgical method, a three-way Foley catheter is inserted through the cervix and into one of the two horns in the cow's uterus. 30/ One channel of the three-tubed catheter inflates a small balloon which prevents the embryo flushing solution from leaving the uterine horn other than by way of the catheter. The remaining two channels of the catheter are for inflow and outflow of the flushing solution. Approximately 500 milliliters of fluid is used to flush each uterine horn. The flushing solution is allowed to drain from the catheter into a collecting dish. The embryos are then examined for normal development. Unfertilized eggs are discarded, along with abnormally developed embryos. "The most consistently useful sign of the embryo's chances of establishing a pregnancy was its stage of development; the less advanced the embryo, the less its chances of surviving." 31/ The average superovulated female produces about ten eggs. Seven of the ten are usually healthy enough to transfer, producing about four calves. 32/ 33/

30/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 352.

31/ Betteridge, K. J. Methods of Assessing the Viability of Embryos. In Betteridge, K. J., ed. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 24.

32/ Caras, Breeding A Revolution, p. 92.

33/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 354.

Embryo Culture/Storage/Cryopreservation

It is sometimes necessary to store embryos for several hours or several weeks or even months before transferring them to recipient females. For short-term storage of less than 24 hours, the embryos are kept at 37 degrees C in a pH buffered salt solution. ^{34/} For periods longer than 24 hours, the temperature is lowered, and the culture media is usually more complex, containing carbohydrates, amino acids, vitamins and nucleic acid precursors. Bovine serum albumin or heat-inactivated blood serum may also be added. ^{35/} "Pregnancy rates for embryos cultured up to 24 hours are similar to those of embryos cultured for only a few hours, but there may be more subsequent embryonic death after transfer of embryos cultured for 24 hours or more. For commercial purposes, embryos are usually kept in vitro for less than 10 hours." ^{36/} "Embryos from cattle, sheep and goats tolerate cooling to 0 degrees to 10 degrees C for several days without substantial loss of viability, but pig embryos do not survive cooling below 15 degrees C." ^{37/} Embryos may also be stored in the oviducts of living rabbits for 2 to 4 days. They continue to develop normally and have high pregnancy rates after they are transferred to the recipient animal.

For longer periods of time, embryos may be stored indefinitely at -196 degrees C in liquid nitrogen. Cryopreservation or deep-freezing of embryos destroys about one-third of the embryos, "but pregnancy rates of the

^{34/} Seidel, Superovulation and Embryo Transfer in Cattle, p. 352-353.

^{35/} Seidel and Seidel, The Embryo Transfer Industry, p. 56.

^{36/} Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

^{37/} Seidel and Seidel, The Embryo Transfer Industry, p. 55.

other two-thirds are not much lower than those of fresh embryos." 38/ Freezing is sometimes used commercially if embryo recipients are not available.

Estrus Detection/Synchronization

Before an embryo transfer can occur the recipient cow must be in the same stage of the estrous cycle as the embryo donor. If the recipient's cycle is off by more than one day, there is much less chance of a pregnancy occurring. The largest cost of embryo transfer is arranging for the needed number of recipient females and maintaining them in a nonpregnant state. This expense is the major reason why embryo transfer is so much more costly than artificial insemination. 39/

Recipients are made available either by having a large enough herd of females, all at different stages of estrus, or by using injections of PGF₂ or progestogens. There is little difference in pregnancy rates between the two methods. The advantage of the hormone injection method is that it allows for the use of a smaller herd of recipient animals. However, if large numbers of embryo transfers are routinely made, then there is little or no cost advantage to the hormone injection method. In addition, the animals must be given the hormone treatments before the required number of recipients needed for the embryo transfers is known. (Therefore, an advantage to embryo freezing is that the embryos would wait for the recipients instead of vice versa. 40/) "The importance ascribed to the technology of estrus synchronization is best illustrated by the fact that more research effort, in terms of man-years or dollars, has been devoted to this problem by universities, Government agencies

38/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

39/ Ibid.

40/ Ibid.

and private industry over the last decade than any other technology related to reproduction." 41/

No matter which method of estrus synchronization is used, estrus detection is still necessary. Animals are observed two to three times daily for signs of estrus, which include allowing other animals to mount, excessive mucus secretion, nervousness and increased walking. 42/ Various marking devices may be attached to bulls that are physically incapable of breeding. The devices leave paint or grease marks on the backs of cows who allow themselves to be mounted. 43/

Embryo Transfer

Both surgical and non-surgical methods of embryo transfer have been developed. Surgical transfer is required for embryos in the very early stages of development. Surgery is also the only method of embryo transfer in sheep, goats and pigs. 44/ Approximately 50 percent of commercial embryo transfers in cows are surgical. 45/ There are two approaches to the surgical transfer:

1. The mid-ventral incision, which requires general anesthesia, but may be less traumatic to the reproductive system and permits better exposure in some animals; or
2. The flank incision, with local anesthesia, which is faster, less expensive, but has a slightly lower pregnancy rate.

After exposing the reproductive system using a mid-ventral incision, the presence of a corpus luteum on an ovary is determined. The corpus luteum is necessary for

41/ Seidel and Seidel, The Embryo Transfer Industry, p. 55.

42/ Sorensen, Animal Reproduction, p. 235-237.

43/ Ibid., p. 298-304.

44/ Seidel and Seidel, The Embryo Transfer Industry, p. 57.

45/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 333.

the maintenance of pregnancy after the transfer has been performed. 46/ The embryo is then transferred to the uterine horn which is on the same side as the corpus luteum, where survival rates are about three times higher than in the other horn. 47/ A small incision is made through the uterine wall and a glass pipette is passed into the uterus. The embryo is expelled into the uterus by means of a syringe attached to the pipette.

In the flank approach, the presence of an active corpus luteum is determined by rectal palpation of the ovaries. 48/ The flank is surgically prepared and a 15-centimeter incision is made. The horn of the uterus is pulled into the incision and the embryo is inserted as described above. There is an increased risk of infection with this method because of the movements of the cow during surgery. In addition, the optimal site for depositing the egg is not always accessible. However, it is used more frequently by commercial embryo transfer units because it saves time and can be done in the field with a minimum of equipment. 49/

The non-surgical method uses equipment similar to that used for artificial insemination. 50/ The presence of a corpus luteum is determined by rectal examination of the ovaries. The transfer equipment is inserted through the cervix into the appropriate uterine horn and the embryo is expelled into the lumen of the uterus. Care must be taken to prevent infection even with the non-surgical method since the progesterone-dominated endocrine environment

46/ Sorensen, Animal Reproduction, p. 350.

47/ Eladen, R. P. Embryo Transfer by Surgical Methods. In Betteridge, K. J. Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canadian Department of Agriculture, Monograph 16, 1977. p. 27.

48/ Ibid.

49/ Seidel and Seidel, The Embryo Transfer Industry, p. 57.

50/ Seidel, Superovulation and Embryo Transfer in Cattle, p. 353.

makes the uterus especially prone to infection.' In contrast, it is extremely difficult to infect the uterus when cows are routinely inseminated because of estrogen domination at that stage of the estrous cycle." 51/

Possible reasons for the failure of embryo transfers are:

1. loss of the embryo because it adhered to the transfer equipment or was not deposited in the appropriate place in the uterus;
2. excessive trauma to the reproductive tract;
3. infertility of the recipient--"often . . . the only animals available as recipients are ones culled from breeding herds for failure to become pregnant." 52/

Applications of Embryo Transfer

The following seven applications of embryo transfer are currently feasible. 53/

1. Obtain more offspring from valuable females. Superovulation and embryo transfer can be used to increase the number of calves produced by a superior cow.

In dairy cattle, for example, 70-90 percent of female calves must be reared as replacements for animals culled for reasons other than low production. If the replacements were selected from the top 10 percent of the herd by amplifying the reproduction of superior cows by embryo transfer, the rate of genetic gain from the female side could be increased three to four times. The National Cooperative Dairy Herd Improvement Program reported that the 275 embryo transfer donors on record were at the 75th percentile for genetic value for milk production, whereas their 50 progeny by embryo transfer that were old enough to have records were at the 87th percentile. The increased genetic value over the population average that these cows would transmit to offspring was estimated to be 112 kg of milk for the donors and 155 kg for their embryo transfer offspring. 54/

51/ Ibid., p. 353.

52/ Seidel and Seidel, *The Embryo Transfer Industry*, p. 62.

53/ Ibid., p. 63-65.

54/ Ibid., p. 63-64.

2. Obtain offspring from infertile females. Infertility due to age or disease can be circumvented using embryo transfer, since "the uterus often loses the ability to support a pregnancy before the ovary ceases to produce ova." ^{55/}

3. Export and import of livestock. Because of their smaller size, embryos are obviously easier and less expensive to transport than adult animals. In addition, there is less chance of introducing new diseases to the importing area when embryos are used rather than adult animals. Adaptation to the new environment is easier for an embryo than for an adult, since the female recipient of the embryo will impart to it passive immunity to the local diseases.

4. Test for undesirable genetic traits. Superovulation and embryo transfer enable the livestock breeder to determine if an animal carries undesirable genetic traits in a shorter period of time than was previously possible. This is because the two techniques provide the opportunity to test many more offspring than would be possible with natural reproduction.

5. Introduce new genes into pathogen-free herds. The use of "total-confinement units" for animal breeding is becoming more common in the swine industry chiefly because of high labor costs and increased land value. This method also allows for closer supervision of the pig's diet and more effective control of diseases and parasites. ^{56/} However, the introduction of new adult animals from other areas for breeding purposes can potentially expose the confinement-bred animals to diseases for which they do not have immunity. Embryo transfer greatly reduces the risk of disease transmission.

^{55/} Ibid., p. 64.

^{56/} Pond, Wilson G. Modern Pork Production. Scientific American. May 1983. p. 96.

CRS-24

6. Increase the population base of rare or endangered animals. The embryos of rare species can be transferred to similar, but more plentiful species; for example, American bison embryos may be transferred to domestic cattle. ^{57/} Embryo transfer could also be of great benefit to zoos or animal sanctuaries in their efforts to maintain or increase the numbers of captive endangered animals. Animals in different zoos could be bred, "thus increasing breed choices when costs, quarantine problems or trauma make shipping of adults impossible." ^{58/}

7. Research. Embryo transfer is a technique for use in research involving in vitro studies of gametes or embryos.

Economic Impact of Embryo Transfer

Approximately 17,000 bovine embryo transfer pregnancies occurred in 1979, costing breeders about \$20,000,000. ^{59/} "Financial volume for 1979 was estimated as 9,000 pregnancies at \$1,800 each plus 8,000 'in house' or 'on farm' pregnancies at \$500 each. This probably is an under estimate. Costs per pregnancy will vary depending on which costs are ascribed to embryo transfer." ^{60/}

Charges for embryo transfer in cattle can vary from \$300 to \$2,000 per pregnancy; costs for horses are higher, and sheep, goats and swine are considerably lower. ^{61/}

^{57/} Seidel and Seidel, *The Embryo Transfer Industry*, p. 65.

^{58/} *Ibid.*

^{59/} Seidel, *Superovulation and Embryo Transfer in Cattle*, p. 351.

^{60/} *Ibid.*, p. 357, footnote 11.

^{61/} Seidel and Seidel, *The Embryo Transfer Industry*, p. 44.

CRS-25

In addition, there are many indirect costs, such as registration and blood typing fees, advertising, or loss of production by the donor while she is under treatment. When these costs are added up and adjusted for the post-partum salvage value of the recipient and the current net interest cost of money, it is economically justifiable for a breeder in the United States to use embryo transfer if he can obtain an average of about \$2,500 per calf at six months of age, including both bull and heifer calves. . . . Some embryo transfer offspring command high prices. For example, the top-selling animal in a recent National Holstein Convention Sale sold for \$131,000, and the topselling heifer in the 1980 ABS Americana sale sold for \$22,000. 62/

However, the current high costs of embryo transfer cannot be recovered in increased meat and milk production. 63/

From the breeder's point of view, the success of embryo transfer is variable, depending not only on the number of pregnancies, but also on market fluctuations, promotional opportunities, management techniques and the breeder's goals. Nevertheless, breeder's comments and the steady increase in use of embryo transfer services attest to the fact that cattle breeders believe embryo transfer is profitable, both for the improvement of their herds and for sales of breeding stock. 64/

C. SEXING OF SPERM AND EMBRYOS

The sex of a mammal is determined at conception and depends upon whether an egg bearing an X chromosome is fertilized by an X chromosome-bearing sperm or a Y chromosome-bearing sperm. Embryos with two X chromosomes will develop into females; those with an X and a Y chromosome will develop into males. Thus, if X-bearing and Y-bearing sperm could be separated, sex selection prior to fertilization would be possible via artificial insemination.

62/ Ibid., p. 44-45.

63/ Ibid., p. 49.

64/ Ibid.

Several approaches to sperm separation have been attempted, including methods based on differences in mass, electrical charge and motility. ^{65/} Another way to predetermine sex would be differential inactivation of either X- or Y-bearing sperm. Methods of inactivation have been tried include treating semen with hormones, chemicals, altered atmospheric pressure, or antibodies against Y-bearing sperm. ^{66/} Researchers have also tried immunizing the female against sperm bearing the Y chromosome. Unfortunately, at the present time no method of sperm separation or inactivation has been successfully developed. ^{67/}

Another approach to sex selection is embryo sexing, or diagnosing the individual's sex after fertilization. Presumably, after the embryos resulting from a superovulation have been sorted by sex, those of the desired sex would be transferred via the methods previously discussed into recipient females. Genetic Engineering, Inc. (Denver, Colorado) claims to have developed such an approach. ^{68/} Since male embryos carry on their cell surfaces a specific protein antigen, Genetic Engineering, Inc. developed a method of determining the presence of this antigen. Embryos carrying the antigen are males, those that do not are females. The company uses antibodies tagged with a fluorescent dye marker to detect the male-specific antigen.

^{65/} Betteridge, Keith J., W. C. D. Hare and Elizabeth L. Singh. Approaches to Sex Selection in Farm Animals. In Brackett, Benjamin G., George E. Seidel, Jr. and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 111.

^{66/} Ibid.

^{67/} Ibid., p. 111-112.

^{68/} Embryo Sexing: Vive la Difference. Economics, v. 286, Feb., 19, 1983, p. 103.

CRS-27

In mice, this approach works with an 80 percent reliability. But cattle embryos present a problem. The difficulty is that the number of antigen targets on the surface of an embryo varies over time. When the embryo comprises only eight cells--about three days after conception--a large number of antigens are expressed on its surface. Unfortunately, by the time that it can be removed from the cow--about six days after conception--this is no longer so. ^{69/}

For these reasons, the embryo sexing process developed by Genetic Engineering, Inc. is viewed with some scepticism by other recombinant DNA companies and academic researchers.

D. IN-VITRO FERTILIZATION

The technology of in vitro fertilization allows an egg to be fertilized by a sperm outside the body, literally, "in glass". Although in vitro fertilization of ova has been attempted in several mammalian species, including pigs, sheep, and cows, "it has been possible to circumvent the oviduct (the natural site of fertilization) with in vitro fertilization and embryo transfer only in mouse and man." ^{70/}

Infertility in mammals "may reflect disease conditions (1) that result in defect egg production, (2) that prevent fertilization of a normal egg or (3) that prevent development of the embryo following fertilization." ^{71/} In the second and third instances, in vitro fertilization and embryo transfer could be used to alleviate the animal's infertility. These two techniques could also be used when fertilization problems are attributed to the male, for example, because of

^{69/} Ibid.

^{70/} Brackett, Benjamin G. Applications of In Vitro Fertilization. In: Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 142.

^{71/} Ibid., p. 156.

low sperm count in semen caused by non-genetic factors. "In a case study of repeat breeder cows, 40.8 percent fertilization failure was observed. If viable ova could be recovered from such cows, in vitro fertilization should provide the means for overcoming problems of sperm-egg meeting." 72/

E. PARTHENOGENESIS, CHIMERAS, TWINNING AND CLONING

Parthenogenesis is defined as "reproduction without the genetic participation of the sperm." 73/ The fertilization of an egg does not take place. Parthenogenesis occurs commonly in nature, particularly among invertebrates (animals without internal backbones). It occurs naturally in some populations of reptiles, amphibians, and fish. 74/ Although birds are capable of parthenogenesis, it rarely occurs in these animals. Mechanisms of parthenogenesis probably vary from one species to another.

Chimeras are individuals produced by combining parts of one embryo with parts of another. The embryos may be of the same or a different species. The term can also be used to refer to a person who has received a transplant of genetically and immunologically different tissue, such as bone marrow. 75/

Twinning is the production of equivalent structures by division. 76/ Identical twins are formed in this way; they develop from a single fertilized

72/ Ibid.

73/ Markert, Clement L., and George E. Seidel, Jr. Parthenogenesis, Identical Twins, and Cloning in Mammals. In Brackett, Benjamin G., George E. Seidel, Jr., and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 193.

74/ Ibid.

75/ Stedman's Medical Dictionary, 22d Edition. Baltimore, The Williams & Wilkins Company, 1972. p. 237.

76/ Ibid., p. 1346.

egg that very early in development divides into two independently growing cell aggregates.

A clone is a group of organisms which have arisen from a single individual as a result of asexual reproduction. 77/

Parthenogenesis, the formation of chimeras, twinning and cloning, are all asexual ways of creating new individuals. They are of interest to geneticists and developmental biologists who wish to learn more about how the information stored in genes is expressed and how organisms develop from eggs to adults. They are also of interest to livestock breeders who like to produce exact copies of outstanding domestic animals. 78/

Chimeras are used in experiments to answer questions about mammalian development. Mice are commonly used for chimera studies. Thousands of mouse chimeras have been manufactured in a process that begins by flushing 4- to 8-cell embryos from female animals. Once the outer coating (called the zona pellucida) is removed, the embryos' cells are mixed together, cultured for a period of time, then implanted into recipient females. 79/ In 1978, scientists at Yale University succeeded in making chimeras derived not from the usual two aggregated mouse embryos, but from three, thus forming individuals with six genetic parents. 80/

Information derived from studies of organisms formed by parthenogenesis may help scientists to answer fundamental questions about cloning. Parthenogenesis, like cloning, is a method of "selfing." It produces offspring fairly similar

77/ Ibid., p. 260.

78/ Markert and Seidel, Parthenogenesis, Identical Twins, and Cloning in Mammals, p. 182.

79/ Markert, Clement L., and Robert M. Petters. Manufactured Hexaparental Mice Show That Adults are Derived from Three Embryonic Cells. Science, v. 202, Oct. 6, 1978. p. 56.

80/ Ibid.

to, but not genetically identical with, the mother. A major obstacle of both procedures is the activation of development. In parthenogenesis the egg must be activated without sperm. Cells must also be activated in some methods of cloning. ^{81/} Since mammals fail to develop to term via parthenogenesis, scientists reason that there may be unknown biological barriers to successful parthenogenetic development. Such barriers would probably have profound consequences for cloning mammals. ^{82/}

Researchers at Colorado State University have been working on a type of cloning in cattle that is very much like natural twinning. Over the last year and a half, 600 such experiments have been performed. ^{83/} Embryos are flushed from the natural mother about seven days after fertilization. Under a microscope set at a magnification of 200 times, each embryo is cut in half. The two halves are implanted in two recipient cows whose estrous cycles are synchronized with the natural mother's cycle. The procedure has successfully produced two sets of identical twin calves. The two heifers and two bulls are the first registered beef calves to be cloned and born on an American farm. ^{84/} The cattle twinning procedure may allow breeders to produce far more high quality animals than would be possible otherwise.

^{81/} Markert and Seidel, Parthenogenesis, Identical Twins and Cloning in Mammals, p. 182.

^{82/} Ibid.

^{83/} Orr, Richard. Calf Cloning is Breeding Milestone. Chicago Tribune, May 10, 1983. p. A3.

^{84/} Ibid.

F. NUCLEAR TRANSPLANTATIONAmphibians

Nuclear transplantation, (also called cloning by some investigators) was first developed in amphibians (frogs and toads) to test a fundamental question of developmental genetics: do differentiated cells (those that have assumed their mature form and function) retain the full genetic potential of a fertilized egg? Successful nuclear transplantation requires the insertion of a totipotent nucleus (one capable of directing the cell to develop into an entire organism) into a mature egg from which the original nucleus has been removed.

Such experiments in the laboratory of Dr. J. N. Gurdon showed that normal toads can develop from fertilized eggs containing nuclei transplanted from developing (embryonic or tadpole) brain, liver, and intestinal cells. ^{85/} However, further experiments with mature cells indicate that at some point in development, a cell's DNA becomes irreversibly modified so that the full complement of genes can no longer be expressed. The mature cells' nuclei failed to allow the fertilized eggs to develop. ^{86/} Although the nuclear transplantation procedure can be described simply, it is actually quite difficult and requires an unusually high level of manual dexterity and microsurgical skill. ^{87/}

^{85/} Goodenough, Ursula and Robert P. Levine. Genetics. New York, Holt Rinehart and Winston, Inc., 1974. p. 717.

^{86/} McKinnell, Robert G. Amphibian Nuclear Transplantation: State of the Art. In Brackett, Benjamin G., George E. Seidel and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 165.

^{87/} Ibid.

Mammals

Nuclear transplantation in mammals is similar to previously described experiments with amphibians. The necessary requirements include a source of eggs, a supply of nuclei from embryonic cells, a procedure for removing nuclei, and a technique for inserting the nuclei into eggs. The surgically altered egg must be cultured in vitro until it can be transferred to an appropriate host, and the uterus that receives the cultured embryo must be in the proper phase of the estrous cycle. 88/

In 1981, Karl Illmensee of the University of Geneva, Switzerland and Peter Hoppe of the Jackson Laboratory, Bar Harbor, Maine, reported the successful "cloning" of three mice. The mice were produced by the transplantation of nuclei from 3- to 4-day-old embryos into recently fertilized eggs whose own nuclei had been removed. 89/ Newspaper reports that spread in the wake of Illmensee and Hoppe's research article were called misleading by critics who objected to the use of the term "cloning" to describe what they saw as nuclear transplant manipulations. The work, these critics claimed, fell short of actual cloning which they asserted would involve producing a mature mouse from a single cell of another mature mouse. 90/

Controversy still surrounds the Illmensee and Hoppe experiments. A committee at the University of Geneva is investigating other experiments performed by Illmensee for "alleged irregularities in the reporting of research data. . . .

88/ Ibid., p. 176.

89/ Marx, Jean L. Three Mice "Cloned" in Switzerland. *Science*, v. 211, Jan. 23, 1981. p. 375.

90/ Misleading Cloning Reports Circulated. *Chemical & Engineering News*, Jan. 12, 1981. p. 7.

CRS-33

The irregularities . . . involve alterations made in experimental protocols after the experiments were completed." 91/ The experiments under investigation were performed in 1982 and "involve the transplantation of nuclei from cancer cells into fertilized eggs whose own nuclei had been removed." 92/ The experiments under investigation are similar in design to those performed in 1981 with Hoppe in Bar Harbor and described above. According to the vice-rector of the University of Geneva, Illmensee has requested that the committee also review his other work, "which has now come under a cloud." 93/ Attempts to reproduce the 1981 Illmensee and Hoppe experiments in mice "have proved difficult, according to Clement Market, an embryologist at Yale University." 94/ A committee at the Jackson Laboratory recently completed its investigation into the work performed by Illmensee in 1981 while he was a visiting professor at the Bar Harbor, Maine facility. The committee's report indicated that it found no evidence of fraud, but recommended that Hoppe and Illmensee repeat their 1981 experiments since the work has not been able to be verified by other investigators. 95/

Applications

The value of nuclear transplantation, according to many researchers, lies in what the experiments can tell scientists about normal and abnormal development;

91/ Marx, Jean L. Swiss Research Questioned. Science, v. 220, June 3, 1983. p. 1023.

92/ Ibid.

93/ Ibid.

94/ Ibid.

95/ Marx, Jean L. Bar Harbor Investigation Reveals No Fraud. Science, v. 220, June 17, 1983. p. 1254.

CRS-34

it was never designed to be used as a reproductive procedure for domestic animals. 96/ Nevertheless, scientists and livestock breeders are interested in its potential application to livestock reproduction. 97/ Conceivably, producing many copies of a valuable pedigreed animal by transplanting nuclei from its body cells into enucleated eggs would be a valuable tool for livestock breeders. However, nuclear transplantation experiments in mammals have all utilized nuclei from embryo cells and not from adult cells. This means that the exact genetic make-up, or characteristics, of the animal formed from such an experiment is unknown in contrast with an experiment using adult cell nuclei. Despite the opinions of many scientists that transplantation experiments with adult cell nuclei may never succeed, researchers are still intrigued with the theory of cloning. Research in the field will undoubtedly continue. 98/

G. GENE TRANSFER

The transfer of genes from one organism to another takes place each time an egg is fertilized. The process allows new and unique combinations of genetic material to occur, and is probably an important reason for the evolutionary success of sexual reproduction. 99/

Even with careful breeding programs, the transfer of specific genes cannot be strictly controlled. In efforts to learn more about how genes work, scientists

96/ Marx, Jean L. More about Cloned Mice. Science, v. 212, Apr. 10, 1981. p. 145.

97/ McKinnell, Amphibian Nuclear Transplantation, p. 164.

98/ Markert and Seidel, Parthenogenesis, Identical Twins and Cloning in Mammals, p. 196.

99/ Solter, Davor. Gene Transfer in Mammalian Cells. In Brackett, Benjamin G., George E. Seidel and Sarah M. Seidel, eds. New Technologies in Animal Breeding. New York, Academic Press, 1981. p. 201.

CRS-35

have developed methods to control the transfer of selected genes between animals. Recombinant DNA techniques allow scientists to insert a particular gene into a given cell or whole organism and to study how that gene is controlled. 100/

Methods

Two procedures have been developed for transferring specific genes into animal cells. In the first method the desired gene is linked to the DNA of an animal virus. The animal cells are then infected with the virus, which is carrying the new gene. Using this method, scientists have linked a rabbit protein gene (beta-globin, a component of the oxygen carrying molecule hemoglobin) with the DNA of SV40, a monkey virus. Monkey cells were then infected with the recombinant SV40 virus, which enabled the cells to make the rabbit protein. 101/

In the second method, DNA containing the desired gene is identified, isolated and then mixed with animal cells. A small percentage of these cells will internalize the DNA and make use of the new gene. Using this method, scientists at Columbia University College of Physicians and Surgeons were able to correct a genetic defect in mouse cells that lacked the enzyme thymidine kinase (TK). The scientists had in effect actually "cured" the cells of their genetic defect. In another experiment at the University of California, Los Angeles (UCLA), scientists used this method on cells which were subsequently implanted into living animals. In 1980, a team of UCLA scientists inserted the gene for an enzyme, called dihydrofolate reductase, into mouse bone marrow cells. The cells were

100/ Ibid., p. 201-202.

101/ Anderson, W. French and Elaine G. Diacumakos. Genetic Engineering of Mammalian Cells. Scientific American, v. 245, July 1981. p. 106.

then implanted into mice, where they made the enzyme according to the transferred gene's instructions. 102/

The only way a specific gene can be incorporated into every cell of an animal is by introducing the gene into fertilized eggs or very young embryos. 103/ The first successful experiment of this kind was reported in 1977 by Dr. Rudolf Jaenish. 104/ Dr. Jaenish and his associates infected 4-16 cell stage embryos with a virus and then implanted the embryos into the uteri of recipient females. Some of the experimental embryos developed into adults whose reproductive cells had incorporated the viral genetic material. When these adults were mated, the viral genome was transmitted to all their progeny. 105/

In another method, the gene to be transferred is directly injected, with a tiny glass micropipette, into the fertilized egg. Sometimes the DNA is injected into the pronucleus of the fertilized egg. The pronucleus is a sperm that has penetrated the egg cell. At this point in development, the egg naturally accepts new DNA, so it is a logical stage in which to inject foreign genes. However, not all of the adult animals which develop from injected eggs make use of (or express) the transferred gene. Of those that do, not all produce offspring containing the gene. This indicates that only some of the adult animal's cells that developed

102/ Cline, M. J., et al. Gene Transfer in Intact Animals. *Nature*, v. 284, Apr. 3, 1980. p. 422.

103/ Solter, *Gene Transfer in Mammalian Cells*, p. 214.

104/ Ibid.

105/ Ibid.

CRS-37

from the injected egg contain the new gene. ^{106/} Scientists continue to refine these procedures.

Applications

For the most part, scientists used gene transfer to answer fundamental questions about gene control, function, and expression. But as techniques of transferring selected genes between animals of the same or even different species improve, scientists are beginning to speculate about their possible application to animal husbandry. For example, gene transfer could be used to improve valuable livestock traits, such as fast growth or high milk production.

Scientists at the University of Washington, the University of Pennsylvania and the Salk Institute recently succeeded in transferring the gene for rat growth hormone into mice. The resulting mice were twice as big as animals that did not receive the foreign gene. The increased size of the mice was not because the growth hormone gene came from a rat, but because the scientists had attached to the rat gene a second gene which they used to control the level of expression of the rat growth hormone gene. The metal zinc was included in the animal's diet. The zinc activated the second gene, which in turn increased the amount of rat growth hormone made by the rat gene. ^{107/} ^{108/}

^{106/} Marx, Jean L. Still More about Gene Transfer. Science, v. 218, Oct. 29, 1982. p. 459.

^{107/} Williams, Jeffrey G. Mouse and Supermouse. Nature, v. 300, Dec. 16, 1982. p. 575.

^{108/} Marx, Jean L. Building Bigger Mice through Gene Transfer. Science, v. 218, Dec. 24, 1982. p. 1298.

II. HEALTH TECHNOLOGIES

A. VACCINE PRODUCTION

The term vaccine is derived from the Latin word *vaccinus* which means "of or from cows." ^{109/} The first vaccine was made about 200 years ago by Edward Jenner, who injected live cowpox virus into a young boy's arm to protect him against smallpox. Since Jenner's time, the term vaccine has come to encompass any preparation used to stimulate the recipient's immune system to produce protective antibodies against a pathogen (disease-causing organism). These preparations may include killed bacteria; attenuated (weakened) but live viruses; or derivatives of microbes, fungi; and parasites. ^{110/} The use of the term vaccine is sometimes confined to viral preparations and the term bacterin is used for preparations made with bacteria.

All vaccines work in essentially the same manner: they stimulate an immune response against the invading organism. When a vaccine enters the body, certain protein molecules, or antigens, on the microorganisms contained in the vaccine are recognized as "foreigners" by the body's surveillance cells. These cells trigger other cells, called lymphocytes, to produce antibodies against the foreign antigens. Without actually producing symptoms of the disease itself, a vaccine sensitizes the lymphocytes so that they can produce the same antibodies rapidly when exposed to the corresponding disease-causing strain of viruses or bacteria.

^{109/} Webster's Seventh New Collegiate Dictionary. Springfield, G. & C. Merriam Co., 1965. p. 979.

^{110/} Stedman's Medical Dictionary, 22d Edition. Baltimore, The Williams & Wilkins Co., 1972. p. 1366.

Producing the large quantities of pathogens necessary for vaccine manufacture can be difficult and expensive. Despite the fact that virulent viruses and bacteria can evade the body's protective mechanisms and multiply in their hosts, they are often quite difficult to grow in culture outside the body. Sometimes they are even difficult to grow in laboratory animals.

There are two forms of vaccine currently being used by the medical community: attenuated (weakened) live organisms and inactivated (dead) organisms. Using attenuated, live organisms is the preferred method of vaccination because it is more likely to induce long-lasting protection. Attenuated virus vaccines may also be referred to as modified live virus vaccines.

The attenuation process alters the organism so that it will no longer cause disease symptoms. However, the growth characteristics and the surface antigens of the organism must not be changed in the attenuation process. If the organism cannot grow, it cannot infect, colonize, and immunologically sensitize the vaccine recipient. If the antigens are changed, the attenuated organism will not trigger production of the appropriate antibodies, and therefore it would be useless as a vaccine. But attenuated virus vaccines are risky since the microbes can revert, through a genetic change other than the one that weakened them, to a virulent form and actually produce the disease they were meant to protect against. ^{111/}

Vaccines containing killed (inactivated) microbes are generally considered safer than those containing attenuated organisms, but the inactivation process often reduces the ability of the preparation to induce immunity. In addition,

^{111/} President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life. Washington, U.S. Govt. Print. Off., 1982. p. 37.

CRS-40

the inactivation process which uses formalin or formaldehyde can cause unwanted side effects, such as allergic reactions, in recipients. There is also the danger that at least one pathogenic organism has survived the inactivation process and will thereby cause the disease in those individuals who receive the contaminated vaccine. 112/

A further problem lies in the instability of vaccines made from either killed or attenuated organisms. These vaccines must be kept under refrigeration a necessity that limits their usefulness in Third World countries where they are most urgently needed. 113/

So in spite of the advances in health care which have been accomplished by using traditional vaccines, there is still room for improvement. New and improved vaccines can be produced by using the recently developed and continually evolving technology called genetic engineering.

The term "genetic engineering" was coined in 1965 to describe "...what has become to be a wide range of techniques by which scientists can add genetically determined characteristics to cells that would not otherwise have possessed them." 114/ Of particular importance to vaccine production is the genetic engineering technique known popularly as gene splicing. This technique, also referred to as recombinant DNA, was made possible by the discovery, in the early 1970s, of restriction enzymes, proteins that exist in nature as part of the defense system bacteria use to respond to foreign DNA. 115/ DNA (deoxyribonucleic acid) is the molecular basis of heredity for most organisms. 116/

112/ The New Vaccines, p. 43.

113/ The New Vaccines, p. 43.

114/ President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life, p. 8.

115/ Ibid., p. 32.

116/ Webster's New Collegiate Dictionary, 1974. p. 335.

Restriction enzymes, of which about 150 have been identified so far, cut DNA at points precisely determined by the chemical sequence of the molecule. The cut segments may contain several genes or only a part of a gene, (the basic functional unit of inheritance) depending on the spacing of sites recognized by the restriction enzymes.

The segment of DNA removed from a chromosome (a string of genes, composed of DNA and protein) can be inserted into a completely different cell by first attaching it to either a virus or a ring of DNA known as a plasmid. Viruses and plasmids act as vectors, carrying the newly attached DNA segment into another cell, usually a bacterial cell. Escherichia coli, which is ~~normally~~ normally present in the human intestine, is the bacterium commonly used for recombinant DNA studies.

Once inside the bacterium, the new piece of DNA is replicated along with the microbe's own DNA each time the cell divides. After many such divisions, a clone of identical cells is formed. Each cell contains a faithful copy of the original recombinant DNA insert. ^{117/} The bacteria then use their own biological machinery to construct the protein product specified by the inserted gene. Scientists can now use recombinant DNA techniques to make vaccines by allowing bacteria to synthesize the necessary antigens required for the body's response to disease. As mentioned earlier, antigens are the part of the disease-causing microbe which stimulate the protective response of the immune system.

The advantages of vaccines produced by recombinant DNA over either attenuated or killed virus vaccines are numerous. They cannot be contaminated by virulent strains of microorganisms, they are easy to purify, they can be produced in unlimited quantities by rapidly growing bacteria, and they are stable.

^{117/} U.S. Congress. House of Representatives. Genetic Engineering, Human Genetics, and Cell Biology: Evolution of Technological Issues. Washington, U.S. Govt. Print. Off., Aug. 1980. p. 9.

Before recombinant DNA vaccines can be produced, the genes coding for the critical antigens must be isolated. In the case of many disease-causing organisms, this means identifying more than one gene, since there are usually several different strains, each with slightly different antigens. A vaccine effective against pneumonia, for example, would need to contain about 83 different antigens. 118/

An even newer approach to vaccine production uses standard chemical procedures to synthesize small portions of the protein antigen instead of the entire antigen as described above. The segment of a protein antigen that produces the sensitizing response essential to immunity is identified and then used as a vaccine. However, a protein's shape and function is determined by more than just the class of its constituent amino acids and the sequence in which they are linked. A protein's three dimensional structure also is important in determining its action. X-ray crystallography and computer modeling are used to analyze a protein's three-dimensional configuration.

Recently, computer analysis was used to predict the critical portions of the hepatitis B virus surface antigen. Two peptides containing one of these regions have been synthesized. The vaccine prepared from the peptides was injected into laboratory mice. The mice responded as expected--they produced antibodies to the hepatitis B virus. 119/

Synthetic peptide vaccines have some of the same advantages as recombinant DNA vaccines. They avoid using pathogens, so there is no danger of contamination by virulent microbes. Synthetic vaccines are very pure, unlimited quantities can be produced quickly and cheaply, and the preparations don't need to be

118/ The New Vaccines, p. 43.

119/ Zuckerman, Arie J. Developing Synthetic Vaccines. Nature, v. 295, Jan. 14, 1982. p. 98.

refrigerated. But synthetic peptide vaccines are not very potent. Scientists still need to develop peptide carriers which boost the effectiveness of these vaccines. 120/

Producing both the synthetic peptide and recombinant DNA vaccines depends on isolating the critical surface antigens of disease-causing organisms, a task that was difficult and time consuming until the development of another genetic engineering advance--cell hybridization. This procedure, which produces hybrid cells called hybridomas, allow scientists to make highly specific antibodies that can be used as "probes" for antigens that may be useful in vaccine development.

In this procedure, scientists first stimulate research animals (usually mice) to produce antibodies by injecting them with the pathogenic organism against which they are interested in developing a vaccine. The antibody-making white blood cells are collected from the mice and then fused with malignant cells (called myelomas) through a chemical process that dissolves and then regenerates the cells' outer membranes. The resulting hybridomas inherit qualities from both parent cells--the cancer cell's ability to grow and divide rapidly and indefinitely and the white cell's ability to produce antibodies. Each hybridoma is cultured individually and eventually produces a clone of identical cells, all synthesizing the same antibody. This antibody is termed a monoclonal antibody because of its production by a clone of cells descended from a single hybridoma. 121/

The great advantage of monoclonal antibodies is their specificity for a single type of antigen or even a small part of an antigen. Using special

120/ The New Vaccines, p. 46.

121/ President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. Splicing Life, p. 35.

radioactive labeling procedures, scientists are able to detect the antigen to which a monoclonal antibody reacts. That antigen can then be used in vaccine development.

Since pathogenic organisms evolve and become resistant to what once were reliable treatment methods, the production of effective vaccines becomes increasingly important. Genetic engineering and cell hybridization should help meet the need for new vaccines and treatment methods.

The following sections describe the development of several genetically engineered vaccines for a number of diseases which affect agriculturally important livestock.

Viral Vaccines

Foot and Mouth Diseases

The National Academy of Sciences has identified 20 animal diseases for which vaccines should be developed. Foot and mouth disease (FMD) is near the top of that list. ^{122/} Indeed, for the last four centuries, FMD has been one of the world's major animal plagues. ^{123/}

Caused by a virus similar to that responsible for polio, foot and mouth disease affects some 32 species of cloven-hoofed animals, including cattle, swine, sheep, and goats. The infection is highly contagious and severely debilitating, but mortality rarely exceeds 6 percent in any species. Infected animals show fever, weakness, loss of appetite, and blisters on the feet, mouth, and tongue. Abortion, mastitis, and chronic deformities of the feet are common. In dairy

^{122/} The New Vaccines, p. 44.

^{123/} Bittle, James L. et. al. Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence. Nature, v. 298, July 1, 1982. p. 30.

cows, milk production is severely diminished. Animals that survive infection may become carriers of the virus, perpetuating the disease. In rare instances, the virus has even infected people. 124/

At least for now, foot and mouth disease has been eradicated in the United States; the last major outbreak occurred in 1954. 125/ But in many parts of the world the disease is endemic (native), costing farmers tens of thousands of animals and billions of dollars a year. Quarantine of the infected area and slaughter of all infected and susceptible animals in contact with the disease is the method of control in countries where FMD does not normally occur. Some years ago, British authorities had to destroy more than 280,000 animals to contain a major outbreak. Vaccination is used where the disease is endemic. Over 1,000 million doses of vaccine are used each year. 126/

There are several problems with the vaccine that is currently being used. Perhaps most important for disease eradication is the fact that the vaccine cannot offer permanent immunity. Another problem is that the vaccine is unstable and requires refrigeration, which is difficult to provide in some parts of the world where the need for the vaccine is greatest. 127/ Furthermore, foot and mouth disease viruses are very hardy. Some can escape inactivation, creating a vaccine that causes the very disease it was intended to prevent. Recent outbreaks of foot and mouth disease in Europe have been traced to improperly inactivated

124/ Brown, Joan M. Genentech's Foot and Mouth Vaccine Could Find Major Markets after Third World Trials. Genetic Engineering News, Jan./Feb. 1983. p. 11.

125/ Ibid.

126/ Bittle, et al. Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence, p.30.

127/ Abelson, Philip H. Foot-and-Mouth Disease Vaccines. Science, v. 218, Dec. 17, 1982. p. 1181.

CRS-46

virus. ^{128/} The United States Government strictly forbids entry of both FMD viruses and vaccines to the mainland. All U.S. studies of foot and mouth disease are done on Plum Island, about a mile and a half off the coast of Long Island. ^{129/}

An important step in solving the FMD vaccine problem has been taken by the Department of Agriculture (USDA), and Genentech, Inc., a biotechnology company in South San Francisco. Using recombinant DNA technology, Genentech researchers have succeeded in developing bacteria which produce large quantities of foot and mouth disease viral protein. The foot and mouth disease virus is surrounded by a coat containing four major proteins. Only one of them, viral protein three (or VP3), triggers the animal's immune system to produce antibodies against the FMD virus. Since VP3 alone cannot cause foot and mouth disease it was viewed as the perfect candidate for a recombinant DNA-produced vaccine.

The bacterially produced protein has been tested as an FMD vaccine at Plum Island by USDA and Genentech researchers. Two injections of the preparation, each containing 250 milligrams of VP3, were given to cattle and swine. The vaccinated animals showed a good antibody response and remained disease-free after exposure to foot and mouth virus. Field trials of the vaccine will begin in Argentina and Columbia, where FMD is endemic, in late 1983. The vaccine will be marketed by International Minerals and Chemicals (IMC), Chicago. ^{130/}

The recombinant DNA vaccine has two major advantages over traditional preparations. First, the vaccine is safe because it does not contain a virus;

^{128/} Bittle, Protection Against Foot-and-Mouth Disease by Immunization with a Chemically Synthesized Peptide Predicted from the Viral Nucleotide Sequence, p. 30.

^{129/} Brown, Genentech's Foot and Mouth Vaccine Could Find Major Markets after Third World Trials, p. 11.

^{130/} Alper, Joseph. Vaccine Research Gets New Boost. High Technology, Apr. 1983. p. 62.

CRS-47

it merely contains noninfective viral protein. Second, the new vaccine is stable and it can withstand a temperature of 100° C, the boiling point of water. 131/ However, the USDA/Genentech vaccine was made from only one strain of foot and mouth virus. A comprehensive vaccine that protects against all 30 foot and mouth disease viral strains, or at least the major ones, awaits further research. According to one estimate, a recombinant DNA vaccine against foot and mouth disease may not be on the market before the mid-1980s. 132/

Rabies

Rabies is caused by a neurotropic virus (one with an affinity for the nervous system) that may affect all species of warm-blooded animals, including man. The frequently fatal infectious disease is transmitted almost exclusively by the bite of carnivorous animals, such as dogs, cats, raccoons, wolves, foxes, bats, and skunks. Rabies symptoms are those of profound nervous system disturbance--excitement, aggressiveness, and madness followed by paralysis and death. 133/

In 1885, Louis Pasteur developed the first rabies vaccine, using dehydrated spinal cords from rabies-infected rabbits. Today, rabies virus can be cultured in vitro (in laboratory culture systems), concentrated, and inactivated to produce a vaccine that is more potent and more easily tolerated than Pasteur's. 134/

Despite treatment advances, rabies is still a worldwide problem. In the United States, mandatory vaccination programs have nearly eradicated rabies

131/ Abelson, Philip H. Foot-and-Mouth Disease Vaccines. Science. p. 1181.

132/ The New Vaccines. Chemical Week, Nov. 24, 1982. p. 45.

133/ Stedman's Medical Dictionary, 22d Edition, p. 1057.

134/ Yelverton, Elizabeth, et al. Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli. Science, v. 219, Feb. 11, 1983. p. 614.

in dogs, but the disease is still widespread in wild animals. In South America, the death of cattle infected by rabid vampire bats costs farmers more than \$28.6 million every year. 135/ 136/

The currently available rabies vaccine has many of the problems already discussed in conjunction with inactivated and attenuated preparations. Several research groups have been actively working on a virus-free rabies vaccine. The gene for a rabies antigen was first isolated from rabies virus at the Wistar Institute in Philadelphia. 137/ Scientists had previously determined that this antigen, a viral surface protein, was capable of stimulating the immune system to make antibodies against rabies.

Researchers at Transgene, a French genetic engineering company, and Genentech, a U.S. firm, have independently placed the gene for this antigen into Escherichia coli, enabling the bacteria to synthesize the rabies antigen. 138/ 139/ This bacterially synthesized rabies antigen will react, in in vitro studies, with rabbit antibodies against rabies, indicating that the antigen will be useful in eliciting the necessary immune response in whole animals. A vaccine prepared from the synthesized rabies antigen could be administered orally, by placing it in food baits, thus preventing rabies in wild animals. 140/

135/ Callas, J. J., G. C. Poppensiek and D.H. Ferris. Surveillance and Control of Virus Diseases: Americas. In Gibbs, E. P. J., ed. Virus Diseases of Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 56.

136/ Yelverton, et al. Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli, Science, p. 614.

137/ Transgene Produces Surface Protein of Rabies Virus. Genetic Engineering Letter, Aug. 10, 1982. p. 3.

138/ Ibid.

139/ Chen, A. Biosynthesis of a Rabies Vaccine, Science News, v. 123, Feb. 12, 1983. p. 103.

140/ Yelverton, et al., Rabies Virus Glycoprotein Analogs: Biosynthesis in Escherichia coli, p. 614.

Scientists have cautioned that they have not yet achieved a genetically engineered rabies vaccine. More tests of further purified viral antigens are needed. A commercial vaccine may not be available until 1986.

Parvovirus

Parvovirus infects a variety of hosts including mink, cats, dogs, cattle, swine and humans. Disease symptoms can include fever, dehydration, inactivity, diarrhea, and vomiting. An acute epidemic of canine parvovirus occurred in the United States during the summer of 1978, and a shortage of vaccines resulted. ^{141/} Although the shortage of canine vaccines has been alleviated, a safer more effective product is still required and could possibly be developed through genetic engineering.

Porcine parvovirus is an important cause of reproductive loss in swine. The infected sow remains healthy but transplacental infection of pig fetuses during the first half of gestation can lead to embryonic death and resorption or mummification, with few surviving normal fetuses. ^{142/} Bovine parvovirus appears to be an important contributor to calf losses from neonatal diarrhea and abortion. The virus has been recovered from feces of normal calves and calves with diarrhea, as well as from aborted fetuses. ^{143/ 144/} Vaccines have not been developed for

^{141/} Siegmund, Otto H. and Clarence M. Fraser, eds. The Merck Veterinary Manual: A Handbook of Diagnosis and Therapy for the Veterinarian, 5th ed. Rahway, N.J., Merck and Co., Inc., 1979. p. 305-306.

^{142/} Derbyshire, J. B. Virus Diseases of Pigs. In Gibbs, E. P. J., ed. Virus Diseases of Food Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 185-186.

^{143/} Kahrs, R. F. Virus Diseases of Cattle. In Gibbs, E. P. J., ed. Virus Diseases of Food Animals, v. 1: International Perspectives. New York, Academic Press Inc., 1981. p. 148.

^{144/} Kahrs, R. F. Viral Diseases of Cattle. Ames, IA, The Iowa State University Press, 1981. p. 183.

porcine or bovine parvovirus at the present time. However, two genetic engineering companies (Applied Molecular Genetics, or Amgen, located in Thousand Oaks, CA and Molecular Genetics in Minnetonka, Minnesota) are working on a porcine parvovirus vaccine. 145/

Bovine Viral Diarrhea

Bovine Viral Diarrhea, BVD (also known as Mucosal-disease complex), is an infectious disease of cattle characterized by ulcerations of the mouth, pharynx, esophagus, stomach and intestines. The infection is usually accompanied by fever and severe diarrhea, and is also associated with congenital arthritis, abortion and defects of the brain in young calves. 146/ Acute, chronic and subclinical BVD can occur, with the acute cases usually being fatal. 147/ Subclinical BVD can predispose the animal to other infections. Because cattle with the acute systemic form usually die, and serious chronic cases are usually culled, it is the subclinical infections that are followed by abortion or the birth of calves with congenital anomalies which cause the major economic impact of BVD. 148/

Both inactivated (killed) and attenuated (modified-live) BVD vaccines are currently available. 149/ The attenuated virus vaccine is not approved for use

145/ Genetic Technology News, v. 3, no. 6, June 1983, p. 3.

146/ Siegmund and Fraser, The Merck Veterinary Manual, p. 266.

147/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 90-91.

148/ Ibid., p. 91.

149/ U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. Veterinary Biological Products: Licensees and Permittees. Issued Jan. 1983. p. 13-14.

in pregnant cattle because it can induce abortion. This vaccine has also been associated with post-vaccination mucosal disease. ^{150/} Genetic engineering companies have begun work on a recombinant DNA vaccine for BVD.

Papillomavirus

Papillomavirus infection produces warts in a variety of animals including rabbits, cows, pigs, dogs, horses, goats and humans. The warts commonly occur on the head, neck and shoulders, and occasionally on the back and abdomen. The venereal form of the wart can be a serious problem in young bulls and can cause difficulties in calving for heifers when it affects the vaginal mucosa. ^{151/} "Economic loss occasionally results when lesions become generalized and excessive, penile warts interfere with breeding, or warts on teats interfere with milking or predispose to mastitis" ^{152/} The presence of warts can cause the reduction of hide value, and the rejection of cattle for export or for participation in shows. Warts occur most commonly in young cattle and usually disappear within one year. Commercial vaccines provide partial protection if administered repeatedly prior to infection. ^{153/} Molecular Genetics (Minnetonka, Minnesota) is developing a bovine papilloma virus vaccine using recombinant DNA technology. ^{154/}

^{150/} Kahre, Viral Diseases of Cattle, The Iowa State University Press, p. 101-102.

^{151/} Siegmund and Fraser, Merck Veterinary Manual, p. 251.

^{152/} Kahre, Viral Diseases of Cattle, The Iowa State University Press, p. 121.

^{153/} Ibid.

^{154/} Genetic Technology News, June 1982, p. 4-5.

Rift Valley Fever

Rift Valley Fever (RVF) is a mosquito-borne viral disease that affects sheep, goats, camels, cattle, buffaloes, antelopes, rodents and humans. RVF is characterized by a short incubation period, fever, hepatitis, death in young animals and abortion in pregnant animals. ^{155/} The disease is confined to Africa but has the potential to spread to other areas in the world. It is named for the Rift Valley in Kenya, where it was first discovered. Mortality rates approach 90 percent in lambs. Adult sheep and cattle are less seriously affected but calves may experience high death rates. ^{156/} Outbreaks of RVF are usually sudden and spread rapidly during the rainy season when mosquitos are active. Current control methods include spraying or using other mosquito control procedures and vaccinating animals with live virus preparations which must be administered annually. Pregnant animals are vaccinated with killed virus preparations. Genetic engineering companies are attempting to develop a vaccine against Rift Valley fever.

Bluetongue

Bluetongue (BT) is an insect-transmitted viral disease affecting sheep, cattle, goats and wild ruminants. ^{157/} BT is principally a disease of sheep,

^{155/} Siegmund and Fraser, Merck Veterinary Manual, p. 437.

^{156/} Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 270.

^{157/} Siegmund and Fraser, Merck Veterinary Manual, p. 251.

but cattle can also become infected and are thought to be carriers of the disease. The BT virus is transmitted by gnats and is not spread by direct contact between animals. The name bluetongue is derived from the blue discoloration of the tongue which is sometimes observed in sheep but rarely seen in cattle. BT infections in sheep are characterized by fever, nasal discharge, mouth sores, swollen blue tongue and lameness. In cattle, BT infections are usually subclinical with inapparent symptoms, but a rare, fatal, usually sporadic disease has been associated with BT infections in cattle. 158/

Because cattle are thought to be carriers for the disease in sheep, some sheep-producing nations prohibit the importation of cattle or bovine semen from BT-infected areas. 159/ 160/ The virus which causes BT is present in many Southern and Western States. 161/ The disease costs the U.S. livestock industry millions of dollars annually due to loss of sales of cattle, semen and embryos to overseas markets. 162/ Control of BT is difficult because of its complex epidemiology. Vaccines are not currently available for cattle. Attenuated virus vaccines are available for use in sheep; killed virus vaccines are being developed. Researchers at the USDA Plum Island Animal Disease Center are developing a recombinant DNA BT vaccine. A viral coat protein

158/ Kahrs, Viral Diseases of Cattle, The Iowa State University Press, p. 71.

159/ Ibid.

160/ Genetic Technology News, v. 3, Feb. 1983, p. 6.

161/ Genetic Engineering Letter, v. 3, Jan. 10, 1983, p. 2.

162/ Genetic Technology News, v. 3, Feb. 1983, p. 6.

of one BT strain has been identified and efforts are now underway to clone its corresponding gene. 163/ 164/

Transmissible Gastroenteritis

Transmissible Gastroenteritis (TGE) is a viral disease of swine characterized by profuse diarrhea and vomiting. TGE rapidly spreads through swine herds causing very high mortality in piglets under two weeks of age due to dehydration. In older animals, the disease is less severe with much lower mortality, but lactating animals may be severely affected. 165/ Outbreaks of TGE usually occur in winter and early spring. The disease is thought to be transmitted by infected swine which shed the virus for up to eight weeks after infection. It may also be spread through contact with infected cats, dogs, foxes, or starlings. 166/ The virus is readily transmitted through feces. TGE may also be contracted via airborne virus if the animals are kept in confined areas. The virus can be destroyed by sunlight and heat but may remain infective for years when frozen. 167/

There is no specific treatment for TGE infections. Modified live virus vaccines for the control of the disease have been of limited value. 168/

163/ Ibid.

164/ Genetic Engineering Letter, v. 3, Jan. 10, 1983, p. 2.

165/ Sigmund and Fraser, Merck Veterinary Manual, p. 293.

166/ Derbyshire, Virus Diseases of Pigs, p. 189.

167/ Sigmund and Fraser, Merck Veterinary Manual, p. 293.

168/ Derbyshire, Virus Diseases of Pigs, p. 189.

Applied Molecular Genetics (Amgen) is in the process of developing a TGE vaccine using recombinant DNA techniques. ^{169/}

Bacterial Vaccines

Colibacillosis

Colibacillosis, or "scours," is caused by a pathogenic strain of Escherichia coli which colonizes the intestines of piglets and calves shortly after birth. The bacterium attaches itself to the animal's intestinal wall with tiny projections, called pili, and then produces a lethal toxin. ^{170/} The disease causes extensive losses in newborn calves and pigs, killing about 10 percent of the animals. ^{171/} Conventional vaccines are available but they are not very effective or long lasting and tend to vary in quality.

The very first products to be made using genetic engineering and marketed were vaccines for porcine and bovine scours developed by Intervet, a subsidiary of Akzo, the Dutch chemical and textile company. ^{172/} Intervet's vaccine is composed of the protein which forms the pili of the pathogenic bacteria. The protein, called K88 in the case of pigs and K99 for cattle, is manufactured by genetically engineered bacteria. When the vaccine is injected into a pig or cow, the animal will make antibodies against the pili protein which prevent the attachment of pathogenic E. coli. Because the

^{169/} Amgen 1983 Company Prospectus, p. 16.

^{170/} First Genetically Engineered Product on Sale. *New Scientist*, May 6, 1982. p. 336.

^{171/} Ibid.

^{172/} Ibid.

infection occurs very rapidly, the vaccine is given to pregnant sows or cattle, who pass the antibodies to their nursing young via their milk.

Cetus Corporation (Berkeley, California) has developed a porcine scours vaccine using the same approach as Intervet, described above. The vaccine will be marketed by Norden Laboratories (Lincoln, Nebraska) which is a subsidiary of SmithKline Beckman (Philadelphia, Pennsylvania). ^{173/} Cetus Corp. is also working with TechAmerica (Elwood, Kansas) to develop a scours vaccine for calves. ^{174/} Salsbury Laboratories, a U.S. subsidiary of Belgium's Solvay Group, is marketing a porcine scours vaccine called EcoBac, developed by Richard E. Isaacson of the University of Michigan and its newly established Center for Molecular Genetics. ^{175/} ^{176/} The vaccine has been approved by USDA, and Salsbury Laboratories has started manufacturing the vaccine at its Charles City, Iowa plant. ^{177/}

In contrast to the vaccines discussed above, an oral monoclonal antibody has been developed by Molecular Genetics, Inc. (Minnetonka, Minnesota) to prevent scours in newborn calves. ^{178/} The antibodies are given to the animals in their routine feed supplements and are made using cell hybridization (hybridoma) techniques, as previously discussed. Since the calf begins producing its own antibodies three days after birth, the animal is susceptible to

^{173/} Dwyer, Paula E. Fildes Shifts Cetus Policies as New Vaccine is Launched. *Bio/Technology*, v. 1, June 1983. p. 313-314.

^{174/} *Genetic Technology News*, v. 3, Feb. 1983, p. 4.

^{175/} *Ibid.*, p. 3-4.

^{176/} *Genetic Engineering News*, v. 3, Mar./Apr. 1983, p. 4.

^{177/} *Genetic Technology News*, v. 3, Feb. 1983, p. 3.

^{178/} *Genetic Technology News*, v. 2, Aug. 1982, p. 7.

CRS-57

scours only during the first two days of life. ^{179/} The product developed by Molecular Genetics, Inc. lasts somewhat longer than two days and therefore is able to keep the animal from developing the disease. Following clinical trials, the product was approved for sale in Canada and will be marketed there by Vetrepahm (London, Ontario, Canada). ^{180/}

Mastitis

Mastitis, or inflammation of the mammary gland, is a complex disease caused by a variety of infectious agents, and therefore is difficult to treat. According to information returned in the October 1981 survey of dairy farmers conducted by the National Dairy Herd Improvement Association, 95 percent of dairy farmers treat 25 percent of their milking herd for mastitis annually and spend over \$500.00 per farm for mastitis treatments alone. This figure does not include the cost of veterinary service, mastitis screening, loss of income from reduced milk production and culling due to mastitis. It is estimated that in 1981, the dairy industry lost over \$1 billion because of mastitis-related decreases in milk production and discarded milk.

At the present time, mastitis is treated by keeping the animal clean and by using antibiotic and anti-inflammatory drugs. Researchers are developing preparations of monoclonal antibodies to induce passive immunity against the pathogens found most often in association with mastitis. Monoclonal antibodies could also be used in the diagnosis of this disease, resulting in early treatment and decreased milk loss. Such immunotherapeutic agents would probably not be available before 1985.

^{179/} Alper, Vaccine Research Gets New Boost, p. 62.

^{180/} Genetic Technology News, v. 3, Apr. 1983, p. 8.

Protozoal/Parasitic Vaccines

A parasite is an organism that lives on or in another species, obtaining food and shelter at the host's expense. Normally, parasites cannot exist without a host. Parasitic animals include many protozoans, such as Plasmodium which causes malaria, flukes, tapeworms and other flatworms, and many roundworms. Protozoal parasites are an important cause of disease and death in humans and domestic animals. Millions of acres of land in Africa cannot be used for cattle grazing because of infestation with tick-borne diseases, such as trypanosomiasis. Tropical and subtropical hemoparasites, which infect the blood, helminthic parasites (worms) and intestinal protozoa, such as coccidia, cause extensive losses in livestock and poultry.

A major problem in developing immunotherapeutic agents to prevent parasitic diseases is the complexity of the parasitic organism's life cycle. Each stage of a parasite's life cycle may be passed in a different host or carrier, making the organism difficult to study in laboratory cultures. Furthermore, many parasites go through extensive antigen changes between each life cycle stage.

Trypanosomes, for example, have at least 104 different antigens. While researchers have begun work on a recombinant DNA malaria vaccine, more basic research on the biology of parasites is needed before practical vaccines can be produced for some of the other parasitic diseases discussed below.

Coccidiosis

Coccidiosis is a parasitic disease caused by one or more species of host-specific protozoans, called coccidia, which infest the host's digestive tract and destroy its inner lining. The disease is relatively common in many animals, and is a serious disease problem in cattle, sheep, goats,

chickens and turkeys. 181/ Infection of the host animal results from ingestion of infective oocysts, the encapsulated intermediate stage in the life cycle of some parasites. Oocysts enter the hosts environment when they are discharged in the feces of an infected animal. 182/ Following ingestion, the infective organisms are released from the oocyst and invade the inner lining of the host's intestines.

Vaccines for the prevention of coccidiosis have not yet been developed. Clinical infections are self-limiting and subside within a week if continuous reinfection does not occur. Medication slows or inhibits reinfection and therefore may shorten the course of infection, reducing the discharge of oocysts, alleviating hemorrhage and diarrhea, and decreasing the chance of secondary infections and mortality. 183/

Trypanosomiasis

Sleeping sickness, or trypanosomiasis, is caused by parasitic protozoans, called trypanosomes, which infect the blood of man and many domestic animals. Trypanosomes usually pass through an intermediate host, such as a leech or tick, before infecting vertebrates. 184/ The African cattle industry loses \$5 billion annually because large areas of land are infected with trypanosomiasis and cannot be used for cattle grazing. 185/ Since this protozoan has evolved a complex set of antigens which appear sequentially during its lifecycle, attempts

181/ Siegmund and Fraser, Merck Veterinary Manual, p. 458.

182/ Ibid.

183/ Ibid., p. 461.

184/ Ibid., p. 420.

185/ Alper, Joseph. Vaccine Research Gets New Boost. High Technology, Apr. 1983. p. 62.

CRS-60

to use trypanosome antigens as a basis for a genetically engineered vaccine may prove to be difficult. Genetic engineering techniques will be useful in basic research studies designed to learn more about gene regulation in these parasites. Until scientists discover which antigens are significant and determine the types of response needed to impart immunity, recombinant DNA technology cannot be applied directly to the disease itself.

Malaria

Organisms belonging to the genus Plasmodium have a worldwide distribution in reptiles, birds and mammals; 22 species have been described as affecting primates alone. ^{186/} There are an estimated 150 million new human cases of malaria per year. ^{187/} The World Health Organization estimates that 107 countries are affected and that about half the world's population is exposed to the risk of infection. ^{188/} The disease has a very high mortality rate in children; approximately one million children in Africa die from malaria each year. ^{189/}

Malaria is transmitted by the bite of infected Anopheles mosquitoes. Anti-malarial drugs are available, but the emergence of drug resistant

^{186/} Warren, McWilson. Malaria. In Hulbert, William, William F. McCulloch and Paul R. Schnurrenberger, eds. Disease Transmitted From Animals to Man, 6th edition. Springfield, Ill., Charles C. Thomas, Publisher, 1975. p. 789.

^{187/} Davidson, George. Who Doesn't Want To Eradicate Malaria? *New Scientist*, Dec. 16, 1982. p. 732.

^{188/} Ibid.

^{189/} Priorities in Biotechnology; Research for International Development. Proceedings of a Workshop, July 26-30, 1982. Washington, D.C., National Academy Press, 1982. p. 79.

Plasmodium in Southeast Asia and South America pose a major health threat. Control of the disease is also being hampered by insecticide-resistant mosquitoes. Vaccines against malaria are not currently available. Scientists at New York University are working on a recombinant DNA-derived malaria vaccine. The research is being funded by the World Health Organization and the U.S. Agency for International Development. 190/

Babesioses

Babesioses, which resembles human malaria, is caused by tick-borne parasites of the genus Babesia which infect and develop within the red blood cells of mammalian hosts. It is a significant disease problem in domestic and wild animals wherever tick-vectors are present, but is a particular problem in the tropics. 191/ Babesioses has also been reported as occurring in man. 192/ The disease is characterized by malaise, fever and anemia. When the central nervous system becomes infected, mania and loss of muscle coordination, followed by coma or death can result. 193/

In endemic areas, animals often become exposed to the parasite in the first year of life. It is thought that they are protected during the first year by maternal antibodies transferred via milk (for about two months), or

190/ Rights Row Clouds Malaria Breakthrough. New Scientist, Dec. 23-30, 1982. p. 781.

191/ Siegmund and Fraser, Merck Veterinary Manual, p. 426.

192/ Wescott, Richard B. Piroplasmosis. In Hubbert, William T., William F. McCulloch and Paul R. Schnurrenberger, eds. Disease Transmitted From Animals to Man, 6th edition. Springfield, Ill., Charles C. Thomas, Publisher, 1975. p. 799.

193/ Seigmund and Fraser, Merck Veterinary Manual, p. 429.

CRS-62

by a temporarily elevated innate resistance. One exposure to Babesia parasites during this period protects most animals from subsequent clinical attacks. Losses to livestock herds (cattle, sheep, goats) occur when: animals escape exposure during the first year, and therefore are not immune; when susceptible animals are moved to endemic areas; or when the parasite-infected ticks move to previously uninfested areas. 194/

Live virus vaccines are used in Australia and parts of South America to control the disease. 195/ Babesiosis has been eliminated in the United States through tick eradication programs, however, resistant ticks now limit the usefulness of this method. 196/ International Minerals and Chemicals, a Chicago company, is sponsoring research at the University of Illinois on vaccines effective against bovine and canine babesiosis. 197/ Dr. Miordag Ristic, a veterinary scientist at Illinois, found that when Babesia enters a red blood cell, it sheds substances called glycoproteins which are essential for the infection process. Glycoproteins are proteins which have attached carbohydrate (sugar) groups. By themselves, the glycoproteins cannot elicit an immune response, but when combined with the carrier saponin, a plant extract, they make very good vaccines. The bovine vaccine has passed preliminary tests, and field trials in Venezuela and Australia are already under way. 198/

194/ Seigmund and Fraser, Merck Veterinary Manual, p. 426-427.

195/ Ibid.

196/ Alper, Vaccine Research Gets New Boost, p. 62.

197/ Ibid.

198/ Ibid.

CRS-63

B. INTERFERONS

Cells in culture and in live animals produce substances called interferons in response to infection by live or inactivated virus. Interferons are small proteins which induce a state of resistance against an invading virus. They are also able to "interfere" with viruses other than the one that evoked their production, but are usually only effective on cells of the species which have produced the interferon. 199/ It is thought that these natural body products might be useful in treating cancer and certain viral infections, such as colds.

Research on interferon has been handicapped by the difficulty and expense of extracting and purifying large quantities of the substance. A number of genetic engineering firms, however, have succeeded in cloning and producing several of the many different types of human interferon. Researchers at Genentech (South San Francisco, California) have produced bovine interferon by a recombinant DNA process. The bovine interferon will be tested for its effectiveness in treating viral diseases in cattle. 200/ 201/

Recently, Genentech representatives met with officials of the U.S. Department of Agriculture and the Food and Drug Administration to determine whether their bovine interferon should be regulated as an animal drug by the FDA or as an animal biologic by the USDA. This jurisdictional question must be answered before product regulation or further testing may continue. 202/

199/ Stedman's Medical Dictionary, 22d ed., p. 640.

200/ Genetic Technology News, Apr. 1982., p. 4.

201/ The Livestock Industry's Genetic Revolution. Business Week, June 21, 1982. p. 130.

202/ Jurisdiction Clarification Sought on Recombinant DNA Bovine Interferon. Food Chemical News, Apr. 4, 1983. p. 20.

C. DIAGNOSTICS/MONOCLONAL ANTIBODIES

As discussed in a previous section, monoclonal antibodies, are produced by a clone of cells derived from a single hybridoma cell. Hybridomas are formed by the fusion of a malignant cell (called myeloma) with an antibody-producing cell. Monoclonal antibodies can be used in diagnostic tests to detect the presence of an infection or cancer in blood or body tissue samples. To facilitate this procedure, the monoclonal antibodies are usually tagged with radioactive labels that allow them to show up under certain laboratory conditions. Monoclonal antibodies have been prepared against a number of human pathogenic viruses, bacteria and parasites. ^{203/} Monoclonal antibody-based diagnostic tests are also being developed and used for diseases which affect livestock. Their use will probably increase as techniques are improved, new antibodies developed and costs lowered.

D. ANIMAL HORMONES

Bacteria can also be used to manufacture animal hormones, natural substances that can speed growth, enhance milk production, and increase the efficiency of feed use. The necessary gene (or genes) must be isolated, inserted into a virus or plasmid, and then into bacteria. As the bacteria reproduce, they replicate the inserted gene along with their own genetic material and then use the instructions provided by the gene to manufacture the desired hormone.

^{203/} Nowinski, Robert C., et al. Monoclonal Antibodies for Diagnosis of Infectious Diseases in Humans. Science, v. 219, Feb. 11, 1983. p. 638.

CRS-65

Growth Hormone

Feed additives and drugs that promote animal growth represent a multimillion dollar business. However, feed additives have developed a bad reputation among consumers. DES, for example, has been used as a feed additive and/or subcutaneous animal implant. Other uses of DES have been associated with the development of human cancer. An alternative that may soon be available is growth hormone, a pituitary product that naturally promotes growth.

In the past, using growth hormone has been very expensive, because the hormone had to be extracted from animal pituitary glands. To obtain one gram of bovine growth hormone, for example, required the glands from one thousand cows. ^{204/} However, genetically engineered bacteria can now be used to cheaply and quickly produce large quantities of growth hormone. Such a possibility is already being realized for chicken growth hormone. Applied Molecular Genetics, Inc. (Amgen), a biotechnology firm based in Thousand Oaks, California has succeeded in splicing the chicken growth hormone gene into bacteria. The genetically engineered bacteria have produced enough hormone for testing purposes. ^{205/}

Initial tests of the recombinant DNA growth hormone indicate that it can increase a chicken's growth rate by 15 percent. If further tests prove efficacy, and if the FDA approves the hormone for use as an additive in chicken feeds, the product may be on the market by the end of 1984. ^{206/} Scientists at Amgen have explained that the growth hormone was developed not to make bigger chickens, but to grow normal sized chickens faster. By sending the

^{204/} The Livestock Industry's Genetic Revolution. Business Week, June 21, 1982. p. 130.

^{205/} Biotechnology Struts into the Hen House. Business Week, Apr. 11, 1983. p. 36.

^{206/} Ibid.

CRS-66

sending the birds to market sooner, farmers should be able to save expenses on feed costs. 207/

Monsanto Corp. and Genentech are collaborating on the development of bovine and porcine growth hormone produced by genetically engineered *E. coli*. Tests are underway to assess the safety and efficacy of these products and if successful, they should be on the market by 1990. 208/ 209/ A study at Cornell University indicated that bovine growth hormone could increase milk production in cows by as much as 40 percent with no increase in feed consumption. 210/ More conservative estimates place the increase at around 15 to 17 percent. 211/ Other companies working on bovine growth hormone include Molecular Genetics, Inc., collaborating with American Cyanamid (Wayne, New Jersey) and Collaborative Genetics (Lexington, Massachusetts) with AKZO Pharma (Netherlands). 212/

Prolactin

Prolactin is the pituitary hormone that stimulates the secretion of milk. It may also stimulate breast growth during pregnancy. 213/ Much less is known about the role of prolactin than about the importance of growth hormone in

207/ Orr, Richard. Superchicken: Cloning May Yield Superior Poultry. Chicago Tribune, Apr. 17, 1983. p. 4:6.

208/ Genetic Technology News, Jan. 1982, p. 3.

209/ Genetic Technology News, Aug. 1982, p. 1.

210/ The Livestock Industry's Genetic Revolution. Business Week, p. 130.

211/ Gorewit, R. C. et al. Improved Animal Efficiency Using Pituitary Hormones. San Francisco, Conference on Biotechnology: Commercial and Industrial Applications and Opportunities, 1982. p. 112-113.

212/ Genetic Technology News, Aug. 1982, p. 1.

213/ Stedman's Medical Dictionary, 22d ed., p. 1023.

biological processes. Prolactin may be capable of altering metabolism, directly or indirectly, in several tissues, such as muscle and fat. The result is enhanced growth of lean body tissue. Some studies have linked increases in prolactin with enhanced growth and milk production. ^{214/} As in the case of growth hormone, described above, it is possible to use recombinant DNA technology to synthesize prolactin. Both growth hormone and prolactin could be used to improve the efficiency of meat and milk production.

Hormone Delivery and Slow-Release Technology

An effective method of administering hormones at regular intervals must be developed before they can be used commercially. A study at Cornell University compared three different forms of bovine growth hormone administration in Holstein cows. The hormone was delivered in one of the following forms: daily subcutaneous injections; continuous intravenous infusion throughout the day; or pulsed intravenous infusion six times a day. The study revealed that all three forms of administration were effective in increasing milk yield. According to these results, it is the amount of growth hormone, not the way in which it is delivered, that is important. ^{215/}

Since daily injections and intravenous infusions of livestock would be time consuming and expensive for commercial use, more practical methods of hormone delivery are being developed which will be convenient for the farmer and still effective in enhancing growth and milk production. These methods allow the hormone to be released slowly over a fairly long period of time.

^{214/} Gorewit, et al., Improved Animal Efficiency Using Pituitary Hormones. p. 115-116.

^{215/} Ibid., p. 114-115.

CRS-68

A slow-releasing ear implant is currently being used, and a long-lasting bolus (a very large, soft pill) that slowly releases the hormone in the stomach has been developed.

E. ANIMAL NUTRITION

The ultimate goal of studies on the nutritional needs of agricultural animals is to increase productivity while decreasing feed requirements, thereby enhancing the efficiency with which human food needs are met. Progress being made in improving the nutrient quality of feed grains, developing nutritious additives for animal feed, and altering the intestinal microorganisms of animals (to allow livestock to derive sustenance from ordinarily indigestible matter) is taking science closer to this goal.

Feed Grain Improvement

Perhaps one of the most effective ways of using the new biotechnology for improving animal nutrition will be the improvement of food plants through genetic engineering. Modern crop management, while it has been able to increase plant quality and productivity, has its drawbacks. For example, current management regimens typically use increasing amounts of expensive nitrogen fertilizers, and must often resort to the use of pesticides and herbicides, which can leave toxic residues in the environment. Furthermore, modern plant breeding has decreased the pool of available genes. Without sufficient potential for genetic variation, some plant crops could be completely destroyed should a major disease outbreak occur. It is also possible that modern inbreeding could result in the loss of important genetic traits. 216/

216/ Barton, Kenneth and W. J. Brill. Prospects in Plant Genetic Engineering. Science, v. 219, Feb. 11, 1983. p. 671.

CRS-69

Recombinant DNA technology could provide solutions to some of these problems. Conceivably, it could be used to improve the nutritional quality of feed plants, decrease nitrogen fertilization requirements, and increase plant resistance to environmental stresses (for example, drought and flood) and diseases.

However, less is known about plant genetics on the molecular level than, for example, about viruses and bacteria, and there are fundamental obstacles that must be overcome if genetic engineering is to be successful in plants.

Plant cells are eucaryotic; their cells contain nuclei in which their major store of genetic information is contained. Bacterial cells, generally considered more primitive, are procaryotes; their genetic material, contained in no nuclei, floats freely in their cytoplasm. Further or perhaps consequentially, plants just don't come accompanied by the variety of genetic entities that, as vehicles for gene transport, have made genetic engineering possible on the bacterial level. Preliminary research also indicates that regulation of plant genes may be much more complex than that of bacterial genes. Researchers have recently found that the plant genes considered most desirable to transfer are likely to be part of multigene systems that will be difficult to manipulate. It is not yet clear that just time, money, and the translation of genetic-engineering information and technology from zoology to botany will solve these problems. 217/

Despite these obstacles, many scientists believe that genetic manipulation of plants is possible and even essential given our increasing need for human and animal food. 218/

Current plant genetics research is following several lines, including protoplast formation, cell fusion and regeneration, the development of new plant strains from mutant cells, and the identification of vectors to carry foreign genes into the plant cell nucleus. Cell fusion studies use protoplasts, which are formed by using enzymes to remove the cell walls from plant cells. Protoplasts of two different plant species are then fused, forming

217/ Check, William A. Engineering the Botanical Gene. Mosaic, v. 13, May/June, 1982. p. 19.

218/ DaYoung, H. Garrett. Crop Genetics: The Seeds of Revolution. High Technology, June, 1983. p. 53.

CRS-70

a hybrid which contains the genetic material of both original cells, a mixture that could not take place naturally. Scientists then attempt to generate a whole plant from the hybrid cells. ^{219/} Only a few such fusions have actually succeeded. For example, tomato cells have been fused with potato cells, but the regenerated hybrid plant was sterile, and thus incapable of producing seeds. ^{220/}

Scientists are also trying to create new crops by culturing leaf cell protoplasts which seem to be capable of asexually generating genetic variants. Some of these genetic variants may be useful to plant breeders. It is not known whether the genetic changes which appear are the result of genetic differences in the leaf cells or of the process of forming and culturing the protoplast cells. The cells are allowed to proliferate and regenerate into whole plants that are tested for survival traits and inheritability of their new characteristics. ^{221/}

Early this year, scientists at Monsanto reported the successful implantation of a bacterial gene into plant cells. The Monsanto team placed the bacterial gene for resistance to the antibiotic Kanamycin into petunia, tobacco, sunflower, and carrot seeds. This was accomplished by using *Agrobacterium tumefaciens*, a bacteria which causes crown gall disease (a type of tumor) in many varieties of plants. *A. tumefaciens* contains a plasmid (a circular piece of DNA) which is responsible for the plant tumor producing properties of the bacteria. In the crown gall disease process, the plasmid is released from the bacteria, enters a plant cell and becomes integrated into the plant's DNA. The scientists at Monsanto made use of this property by placing the gene for Kanamycin resistance

^{219/} Solomon, Stephen. Plants that Break the Gene Barrier. Science Digest, Apr., 1983. p. 18.

^{220/} Shepard, James F. The Regeneration of Potato Plants from Leaf-Cell Protoplasts. Scientific American, May 1982. p. 166.

^{221/} Ibid., p. 164.

CRS-71

into the A. tumefaciens plasmid. The plasmid was then inserted into the A. tumefaciens bacteria, and the bacteria was allowed to be mixed with normal plant cells. The incorporation of the Kanamycin-resistance gene from the plasmid into the plant cells was demonstrated by the fact that some of the plant cells were able to survive and grow on media containing Kanamycin, which would ordinarily kill normal plant cells. This work represents the first time an engineered gene has been transferred and expressed in a plant. 222/ Scientists hope to use A. tumefaciens and its plasmid in future studies to transfer agriculturally useful genes which would provide traits such as:

- disease and pest resistance;
- higher yield;
- accelerated growth rates;
- temperature tolerance;
- crops developed for arid regions;
- tolerance to high salt concentrations;
- increased photosynthesis efficiency;
- improved amino acid balance in grain proteins; and
- nitrogen fixing ability.

Feed Additive Production- Amino Acids

Proteins are large, complex molecules consisting of long chains of amino acids. Plants are capable of synthesizing all twenty amino acids required for protein production from simple sugars and nitrogen-containing precursor molecules. Most animals including humans, are unable to synthesize ten of the amino acids which must, therefore, be supplied in the diet. These ten amino acids are usually referred to as the "essential amino acids." Finding better ways of supplying these essential amino acids to agricultural animals is the focus of many research projects.

222/ DeYoung, Crop Genetics, p. 54-55.

CRS-72

Plants store protein, made of amino acids, in their seeds which are often used as animal feed. However, these storage proteins don't always contain all ten essential amino acids. The plant does not need to have a ready supply of all the amino acids, since it can manufacture them for itself. One solution, as mentioned in the list on the previous page, would be to use genetic engineering techniques to improve the amino acid balance of grain proteins. This would be accomplished by inserting the gene for a desired storage protein into, for example, corn plant cells. The much publicized "sunbean" is one such attempt in which a protein gene from a bean plant was inserted into sunflower tissue culture cells. ^{223/} The scientists, however, were unable to induce the mass of "sunbean" cells to form a viable plant.

At the present time, amino acids are added to some livestock feed mixtures. Bacteria are used in fermentation processes to produce essential amino acids such as lysine and tryptophan, which are then added to animal feed. Recombinant DNA technology is currently being used to improve these amino acid fermentations and thus decrease the cost of these feed additives. If this possibility is realized, it could save farmers billions of pounds of feed protein annually. ^{224/}

^{223/} Bean Gene moved to Sunflower Cell. Agricultural Research, Aug. 1981. p. 4-5.

^{224/} Pond, Wilson G. Modern Pork Production. Scientific American, v. 248, May, 1983. p. 102.

Single Cell Protein

An alternative to feed grains, improved or otherwise, is "non-crop" protein provided by single-celled organisms. Single cell protein is the name given to protein materials derived from the controlled fermentation of substrates by microorganisms. ^{225/} Microorganisms have been used for centuries in processes for making cheese and yogurt. Genetic engineering may allow us to use microorganisms on a much larger scale to convert non-food items (for example, sewage, methane, and woodpulp) to palatable, high quality food. The requirements are a cheaper substrate, microorganisms (fungi, algae, yeast, or bacteria), and a mechanical system to mix and aerate the fermenting mass. ^{226/}

One such product, called "Pruteen" is composed of the bacterium Methylophilus methylotrophus. In the Pruteen production process M. methylotrophus is grown in large fermenters using methanol, ammonia and air as raw materials. Other inputs include steam, electricity, gas, phosphoric acid, sulphuric acid and various inorganic salts. Within 36 hours the bacteria have multiplied so that the fermenter contains 40 metric tons of cells. The product can then be dried into pellets which meet the size specifications required by the feed industry. The product Pruteen, like any other dietary protein material, is not a single component but a mixture of proteins, fats, minerals and trace ingredients. The protein level in Pruteen is 73 percent. Tests of Pruteen indicate that it is a safe raw material for use in animal feeding. ^{227/}

^{225/} Waterworth, D. G. Single Cell Protein. Outlook on Agriculture, v. 10, 1981. p. 405.

^{226/} Ibid., p. 405.

^{227/} Ibid., p. 406.

CRS-74

Single cell protein has several advantages over the dietary protein provided in conventional feed grain: supply is uninterrupted by bad weather; the product is consistent; and there are no contaminants. 208/

Manipulation of Intestinal Microflora

It has been estimated that animal feed grains will become less available in the 21st Century than they are at the present time in the United States. 229/ To sustain the current levels of animal production, alternative feed sources will have to be developed. One such source may be cellulose, which is a complex polysaccharide forming the basis of plant fiber. Cellulose is the most abundant organic compound in the world. 230/ Plants containing cellulose grow almost everywhere on earth, and contain as much energy as starchy crops. However, cellulose is not efficiently utilized by animals.

One possible solution would be to change the activity of intestinal microflora (bacteria) of certain agricultural animals. Recombinant DNA technology could be used to alter these natural gut bacteria so that they would make cellulase (the enzyme which cleaves cellulose) thus allowing cellulose to be digested. 231/

228/ Ibid., p. 408.

229/ Wedin, W. F., et al. Feed Production. In Animal Agriculture: Research to Meet Human Needs in the 21st Century. p. 165.

230/ Stedman's Medical Dictionary, 22d ed., p. 249.

231/ Pond, Modern Pork Production, p. 101-102.

CRS-75

CONCLUSION

Breeding livestock for increased productivity is an extremely slow process, especially with species such as cattle which have long generation periods. Most cattle breeders will only be able to work with six or seven generations of cattle. ^{232/} Nevertheless, techniques such as artificial insemination have made a tremendous impact upon, for example, increased milk production in the dairy industry. It is anticipated that the advances in reproductive and health technologies discussed in this paper will accelerate the process of selective breeding for improved traits in the near future.

A majority of these advances in animal sciences are based on discoveries made over the past 10 years in genetics and biomedical research. These discoveries reflect the commitment of serious public support for research in the natural sciences, especially during the last 30 years. Since 1950 approximately \$75 billion from private and public funds have been expended for research and development on health care in the United States, and the annual investment, currently (1981) represents about 0.31 percent of the gross national product. About 60 percent of total funds and an estimated 90 percent of the basic research has been supported by the Federal budget. ^{233/} Although these sums are identified as health care research, it should be noted that many of the new technologies and much of the basic biology of mammalian reproductive systems have evolved

^{232/} Seidel, Superovulation and Embryo Transfer in Cattle, p. 351.

^{233/} Brackett, Benjamin G., George F. Siedel, Jr., and Sarah M. Siedel. Continuation and Implementation of Research. In their: New technologies in Animal Breeding. New York, Academic Press, 1981. p. 257.

CRS-76

from the basic biological research funded by such institutions as the National Institutes of Health and the National Science Foundation as well as the Department of Agriculture.

The continued success of these and other technological advances will depend on the degree of both public and private sector support for research and development in the agricultural sciences and in basic biological research in general.

